Vol. XVI. *April, 1909.* *No. 5.*

BIOLOGICAL BULLETIN

THE NUCLEOLI IN THE SPERMATOCYTES AND GERMINAL VESICLES OF EUSCHISTUS VARIOLARIUS.

KATHARINE FOOT AND E. C. STROBELL.

I. In the resting first spermatocyte of many insects there appears a spherical, chromatic body which invariably stains as intensely as the chromosomes.

McClung ('02) was the first investigator to draw special attention to this structure by his suggestion that it may function as a sex determinant.

A majority of the cytologists who have studied this structure interpret it as one of the chromosomes which persists through the rest stage of the first spermatocyte, and they claim that its presence or absence in a spermatozoön is the determining factor of sex, McClung assuming that its presence in a spermatozoön causes the fertilized egg to produce a male, whereas Stevens, Wilson and others affirm that its presence in a spermatozoön causes the fertilized egg to produce a female, and in those cases where two (a large and a small) chromatin nucleoli are present, the larger is the female-determining, and the smaller, the male-determining factor.

Identifying the chromatin nucleolus as a chromosome enlarges at once the sphere of the problem, involving this structure in the maze of hypotheses and theories associated with the chromosomes. This identification of the chromatin nucleolus as a chromosome is of great importance, because it presumably demonstrates that a definite chromosome retains its individuality throughout the rest stage, and it presents very strong evidence for the theory of the individuality and continuity of the chromosomes — the theory which is the corner-stone of all hypotheses involving the

claim that the chromosomes are the cause rather than the expression of cell activities.

We believe that the evidence we find in *Euschistus*, as well as in *Anasa* ('07), is distinctly opposed to the interpretation that the chromatin nucleolus of the resting first spermatocyte is a phase of one or more of the chromosomes, but we shall reserve the publication of this evidence in *Euschistus* until we can control it by a more complete comparison with other forms. In the present paper we shall limit ourselves to the relatively simple question, is the chromatin nucleolus a structure associated with the male cell only, as claimed by McClung, or is it, as claimed by Wilson, a chromosome received from the egg, which during the rest stage appears in the form of a chromatin nucleolus?

Two chromatin nucleoli unequal in size have been demonstrated by Montgomery ('98, '01, '06) in the first spermatocytes of several varieties of *Euschistus*, including *Euschistus variolarius*, and Wilson ('05) has demonstrated these structures in other Hemiptera, naming them chromosome nucleoli, because he believes them to be chromosomes. In describing them in *Cœnus* and *Lygæus* he says: "Throughout the whole of the growth period in *Cœnus*, and from stage *e* onward in *Lygæus*, at least one of the idiochromosomes can always be distinguished as a compact, spheroidal, intensely staining chromosome nucleolus and frequently both idiochromosomes are distinguishable in this form in all of these stages. . . . It is clear beyond all question that at least the large idiochromosome may retain its identity throughout the whole growth period, with the small idiochromosome the case is not so strong" (p. 389). Of *Brochymena* he says: "There can be no doubt that when only one chromosome nucleolus is present it is to be considered as a bivalent body arising by the fusion or synapsis of the two idiochromosomes" (p. 392).

In the resting first spermatocyte of *Euschistus variolarius* the chromatin nucleolus is the most deeply stained and conspicuous structure in the cell (Photos 1–10). As a rule, only one is present, but quite frequently there are two, sometimes equal, but in most cases, very unequal in size; exceptionally we have found three, or even four, nucleoli in the same nucleus.

We roughly estimated the number of the nuclei with one or

more chromatin nucleoli, by counting 625 cells in one testis, and of these cells 591 showed one nucleolus, 27 two nucleoli, and 7 three nucleoli. These 625 cells represented only a small area of the preparation and we did not repeat the experiment on another testis — the estimate, therefore, offers merely an indication that in a large majority of cases only one chromatin nucleolus is present.

By a comparative study of the spermatocytes and oöcytes of the same form we ought to find an answer to the question, whether the chromatin nucleolus is associated with the male cell only, or whether it has its origin in the oöcyte.

As far as we are aware, the germinal vesicles shown in Plates I., II. and III. are the first that have been demonstrated in any of the Hemiptera heteroptera. A great deal has been written about the spermotocytes and important generalizations have been drawn from the behavior of the chromatin nucleolus in these male cells, but not one word has been said about the corresponding, very important stages in the female, although we have been confidently assured that the chromatin nucleolus in the male has been derived from the egg. If this is true, it would seem at least logical to expect to find some evidence of its presence in the egg at the stage of development corresponding to the stage in which it is so conspicuous in the male cell.

Even if we assume for the sake of argument that the chromatin nucleolus is a chromosome, we still do not avoid the logical expectation of finding it in the oöcytes, for if one of the chromosomes of the spermatocyte possesses the distinguishing trait of persisting through the rest stage in the form of a chromosome nucleolus, and if that chromosome is a chromosome contributed to the male cells from the mother, we should expect to see its distinguishing characteristic manifested in even a more marked degree in the oöcyte. And further, as the chromosome nucleolus of the spermatocyte is claimed to be only one of a pair of chromosomes — its mate being contributed by the egg at the time of fertilization — we should expect to find in the resting oöcyte a pair of chromosomes represented by two univalent, or one bivalent chromosome nucleolus.

In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the

accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes.

II. We find the chromatin nucleolus of the spermatocyte persisting through the entire growth period, Photos 1 to 10. All the resting spermatocytes shown in these photos, are from testes which contain many first spermatocyte metaphases, in which the idiochromosomes show the typical inequality in size. We have selected preparations of the resting spermatocytes showing variations in the relative size of the two chromatin nucleoli (when present) in order to demonstrate their frequent lack of conformity to the size relations of the idiochromosomes. Photo 10 shows a nucleolus persisting until the chromosomes are formed and we have several photographs demonstrating its presence to a still later stage, where the seven bivalents can be counted, but we shall reserve the discussion of these later stages for a subsequent paper.[1]

As the chromatin nucleoli of the first spermatocyte of *Euschistus* persist through the entire growth period, we certainly have a right to expect to find in the egg the equivalent of the large chromatin nucleolus during these same stages, if as asserted it owes its origin to the egg. Again, if the so-called male sex-determinant (the small chromatin nucleolus) persists through the growth stages in the male cell, there seems to be no clear reason why the so-called female sex-determinant (the larger chromatin nucleolus) should not persist through the growth period of the oöcyte.

A study of the oöcytes shown in Photos 11 to 30 demonstrates that in none of these cells can be found a structure resembling in any way the chromatin nucleolus shown in the spermatocytes of Photos 1 to 10. This is clearly demonstrated in the spermatocytes and oöcytes of Plate I. There is no structure in the oöcytes of Photos 11 to 19 which resembles in the least the chromatin nucleolus of the spermatocytes of Photos 1 to 10. The only nucleolus in the oöcytes is a relatively large achromatic structure, first clearly demonstrated in the older oöcytes. It is

[1] We have a number of photographs showing the transition stages between Photos 9 and 10, but lack of space prevents their reproduction on these plates.

clearly seen in the germinal vesicles of Photos 18 and 19, Plate I., and in the germinal vesicles of Photos 20 to 25, Plate II., and in Photos 26 to 30, Plate III. All these preparations show, that as a rule its position is peripheral and this is also true of the principal nucleolus of the germinal vesicles of *Allolobophora* (Photo 31). The absence of a chromatin nucleolus is conspicuous not only in the germinal vesicles of *Euschistus* but in the young oöcytes as well. Compare the young oöcytes of Photos 11 and 12 with the young spermatocytes of Photos 1 to 5.

In none of the oöcytes, from the earliest to the latest stages do we find a chromatin nucleolus, and its absence in the germinal vesicles of *Euschistus* is made more conspicuous by the fact that a chromatin nucleolus is invariably present in the germinal vesicles of *Allolobophora*. If we could find such a structure in the germinal vesicles of *Euschistus*, in addition to the achromatic nucleolus, the advocates of the sex determination hypothesis could justly claim it as convincing evidence in support of their theory of its female origin, even if a demonstration of its relation to one of the chromosomes was lacking.

The absence of a chromatic nucleolus in these germinal vesicles cannot be due to the technique since in this case the more delicate achromatic nucleolus would show some disturbance. This point is exemplified in the germinal vesicle of *Allolobophora* (Photo 31), for in this preparation the pricking has disturbed the large, less chromatic nucleolus, while the small, denser, chromatic nucleolus remains perfectly intact, and this condition holds true for the hundreds of germinal vesicles of *Allolobophora* we have preserved.

It therefore seems fair to assume that the technique is not responsible for the absence of a chromatin nucleolus in the germinal vesicles of *Euschistus*.

In an earlier paper [1] we ventured to call attention to the likeness of the chromatin nucleolus in the egg of *Allolobophora* to the chromatin nucleolus of the first spermatocytes of insects. Compare the chromatin nucleolus in *Euschistus*, Photos 1 to 10, with the chromatin nucleolus in the egg of *Allolobophora*, Photos 31 and 32. In both these forms the chromatin nucleolus stains as

[1] Foot and Strobell ('05).

intensely as the chromosomes, and in both forms we sometimes find one and sometimes two chromatin nucleoli.[1] The comparison may be carried even a step further. We may compare the chromatin nucleolus of *Allolobophora* to the chromatin nucleolus of some forms where it is figured at one pole of the first spindle as an undivided chromosome. The chromatin nucleolus of *Allolobophora* often persists through the first division, and in such cases it is either free in the cytoplasm, near the spindle, or at one pole of the spindle in which position it strongly resembles the undivided chromosome figured in so many forms. Photo 32 shows a section through the first maturation spindle of an egg of *Allolobophora*, and a comparison of the chromatin nucleolus at the upper pole of this spindle, with the chromatin nucleolus in the germinal vesicle of Photo 31 leaves no doubt of the identity of the two structures. The identification is further confirmed by the clear demonstration in this and the adjoining sections of all eleven chromosomes in the equatorial plate of the spindle. If the chromosomes of *Allolobophora* were spherical as they appear in sections of so many forms, we could not so easily identify the chromatin nucleolus, for it stains exactly like the chromosomes. Such a case is described by Arnold ('08) in the spermatogenesis of the Coleopteran *Hydrophilus piceus*. During the rest stage the chromatin nucleolus of this form is easily recognized, but he finds it impossible to differentiate it from the chromosomes during the first prophase, because the chromosomes at this stage are also spherical and both structures stain alike. He is able to identify it again at the first metaphase, where it lies in the cytoplasm near the spindle, or within the spindle at one pole. It persists through the first division, but disappears before the second mitosis.[2] It is clear that in its origin, behavior and time of disappearance the chromatin nucleolus of the spermatocytes of *Hydrophilus* closely resembles the chromatin nucleolus of *Allolobophora*, with the exception that the latter does not invariably persist through the first division. Arnold ('08) supports Moore and Robinson's ('05) conclusion that the chromatin nucleolus of

[1] Foot and Strobell ('05), Photo 115.

[2] Wheeler ('97) figured the nucleolus of the germinal vesicle of *Mysostoma glabrum* persisting through the second cleavage and he says that in some cases it persists through the next stage, and perhaps even later, page 35.

the first spermatocyte is not a chromosome and does not take part in either division. His evidence for this is very convincing, he says, if it is to be interpreted as an accessory chromosome which remains undivided in the first spindle it should divide in the second spindle, and half the second spindles should show one more chromosome than the other half. Arnold finds, on the contrary that the number of chromosomes in the second spindle is as constant as the number in the first. He gives in the following table the results he obtained:

Out of 100 counts of first meiotic:

85 per cent. gave.....15 gemini and the nucleolus.
7 " " " over 15 " " " "
8 " " " under 15 " " " "

Out of another 100 counts of first meiotic:

90 per cent. gave.....15 gemini and the nucleolus.
3 " " " over 15 " " " "
7 " " " under 15 " " " "

Out of 100 counts of second meiotic:

91 per cent. gave.....15 chromosomes.
6 " " " over 15 "
3 " " " under 15 "

The resemblance between the chromatin nucleolus of the first spermatocyte of insects and the chromatin nucleolus in the germinal vesicles of some forms has been noted by Häcker ('07) who says, that in those cases in which the heterochromosome is figured as a cap-shaped mass on a large pale plasmosome, the two structures are extraordinarily like the two kinds of nucleoli in the germinal vesicles of the lamellibranch type. He says: "So kann man sich dem Eindruck nicht entziehen, das z. B. das, 'akzessorische Chromosom' in den ruhenden Spermatocyten von Scolopendra (Blackman, 1905, tab. 2, fig. 10–15) früher einfach als 'Nucleolus' beschrieben worden wäre, und man ist geneigt, sich zu fragen, ob denn in allen vorliegenden Angaben bereits eine reinliche Scheidung zwischen den Heterochromosomen und den echten Nucleolen (Plasmosomen) durchgeführt ist. Es sei mir auch gestattet, darauf hinzuweisen, das die von Wilson u. a. gegebenen Bilder, in welchen ein Heterochromosom als

kappenförmige Masse einem grossen Plasmosom aufgelagert erscheint, eine auserordentliche Ähnlichkeit mit den Befunden in den Keimbläschen des Lamellibranchiaten-Typus zeigen, d. h. in denjenigen Keimblässen, in welchen sich zweierlei Nucleolen von verschiedener Lichtbrechung, Quellbarkeit und Tingierbarkeit befinden."

It is a suggestive fact that in *Allolobophora* we have an hermaphrodite form, and if one is determined to regard the chromatin nucleolus of the spermatocytes as a sex-determinant, some interesting conclusions might be drawn from a comparison of the two types of nucleoli observed in this hermaphrodite form, with the two types characteristic of the male and female cells in *Euschistus*. Compare for example the large nucleolus in the germinal vesicle of *Allolobophora* (Photo 31) with the large achromatic nucleolus of the germinal vesicles of *Euschistus* (Photos 18 to 30), and compare further the smaller chromatin nucleolus of *Allolobophora* (Photos 31 and 32) with the chromatin nucleolus of the spermatocytes of *Euschistus* (Photos 1 to 10).

Even if we could be sure that in *Euschistus* the achromatic nucleolus of the egg cells is not represented in the male cells, and that a striking likeness exists between the male and female nucleoli of *Euschistus* and the two types of nucleoli in the germinal vesicles of *Allolobophora*, we would still be unable to claim from the comparison any results of general significance, for the reason that the observations of many investigators of the spermatogenesis of insects offer contradictions to an assumption that the achromatic nucleolus is associated solely with the female cell — these observers having figured a pale, achromatic nucleolus in the spermatocytes, in addition to the chromatic nucleus characteristic of these cells. As opposed to these facts, however, other investigators have found no structure in the spermatocytes that can be interpreted as a nucleolus, other than the characteristic chromatin nucleolus.[1] These conflicting observations, if equally reliable, compel the conclusion that individual forms may differ as to the absence or presence of an achromatic nucleolus.

[1] In his "Studies on Chromosomes — IV.," *Jour. Exp. Zoöl.*, Vol. VI., No. 1, 1909, Wilson in describing *Syromastes*, does not mention a large pale plasmosome, such as he has described in other forms. In *Pyrrochoris* he states that there are from one to three nucleolar-like bodies, which on account of the staining reactions, he believes to be plasmosomes, page 82.

In *Euschistus* we have not been able to demonstrate its presence at any stage of the growth-period of the spermatocytes. In sections we often find faintly staining areas that might be interpreted as an achromatic nucleolus, but in view of the possibility of artefacts in such preparations, we hesitate to interpret them as true nucleoli, unless we can support the interpretation in our smear preparations. Until this point can be settled we are not justified in drawing any conclusions from the obvious difference in type between the nucleoli in the male and female cells of *Euschistus*, though we have here quite as marked a sexual difference of the nucleoli, as any that has been shown for the chromosomes, a difference that appears in no way associated with the maturation divisions of either germ cell.

In comparing the achromatic nucleolus of the oöcytes of *Euschistus* with the principal nucleolus of *Allolobophora* we find some individual differences between the two. The principal nucleolus of *Allolobophora* is clearly differentiated in the young oöcytes as a small, dense chromatic body, and it can be traced uninterruptedly through the entire growth period of the oöcytes. During this period it increases in size, proportionately to the growth of the nucleus, gradually becomes less dense and less chromatic, and finally, when the germinal vesicle has reached its maximum size the principal nucleolus often appears as in Photo 31. In some cases, however, its dense and chromatic character, distinctive of the earlier stages, persists until the chromosomes are fully formed or again it may so completely disintegrate that only a clear space remains as evidence of its existence.[1]

The achromatic nucleolus of *Euschistus* differs from the principal nucleolus of *Allolobophora* in not being present in the young oöcytes as a small dense chromatic nucleolus. We have been unable to demonstrate any such chromatic body in the young oöcytes of *Euschistus*. In the youngest stages in which a nucleolus is found, it appears as clearly achromatic as when it has reached its maximum growth in the germinal vesicle — compare Photos 13 and 16 with Photos 18 to 30. There are also indications that it is often not formed until after the oöcytes have attained a definite growth, for in such clear preparations as are shown in

[1] Foot and Strobell ('05), Photo 122.

Photos 11, 12, 14, 15 and 17, there is no evidence that such a structure is present.[1]

If the nucleolus in the germinal vesicle of *Euschistus* corresponds to the principal nucleolus of *Allolobophora* it must be conceded that there are marked individual differences between the two, differences quite as marked as those existing between the chromosomes of these two forms.

Individuality and Continuity of the Chromosomes.

The investigators who question the individuality and continuity of the chromosomes urge the necessity for further study of the critical stages bearing on this problem — the stages occurring between the end of one mitosis and the beginning of the next. Meves ('08) holds that during these stages it is impossible to recognize the chromosomes. He says: "Man sucht hier, wie O. Hertwig 1890 geschrieben hat, in den Kern etwas hineinzudemonstrieren, was kein unbefangener Beobachter in seiner Struktur erkennen wird.

Fick ('07) claims, that a study of the rest stages proves that the theory of the individuality and continuity of the chromosomes is untenable.

Tellyesniczky ('07) also makes a strong plea for more thorough and careful work on these stages. We are in sympathy with him in his skepticism concerning the individuality and continuity of the chromosomes, but our results differ from his in some, perhaps unimportant, details — differences which may normally exist in different forms. For example Tellyesniczky ('05) believes that a homogeneous distribution of the nuclear substance throughout the nuclear vesicle precedes every mitosis, and the young spermatocytes of our Photos 1, 4 and 5 lend support to this claim. The young oöcyte of Photo 13 also indicates a diffused condition of the chromatin, but as a rule the chromatin of the young germinal vesicles appears as fine granules often very evenly distributed throughout the nucleus as in Photos 11, 14 and 17. Possibly this granular condition may be an artefact — one phase of a precipitate left after drying, for in some cases the

[1] Among recent observations on this point, Deton ('08) finds in *Thysanozoön Brochii* that the nucleolus is absent in the young oöcyte, first appearing at the beginning of the growth period.

chromatin of the young oöcytes is as homogeneous as that of the spermatocytes of Photos 1, 4 and 5.

We find nothing in these germinal vesicles answering to Tellyesniczky's karysomes, unless he would homologize the aggregations of chromatin shown in Photos 12, 15 and 16 with these structures. These aggregations of chromatin are too numerous to be interpreted as the seven bivalent chromosomes of the later stages, or as their fourteen component univalents. In the germinal vesicle of Photo 15, for example, there are about fifty clumps of chromatic substance. Perhaps these clumps of chromatin correspond more closely to the segregated granules Wassilieff ('07) describes in the nucleus of *Blatta*. These clumps, he says, are too numerous to be interpreted as representing individual chromosomes. Later he finds that they disintegrate and are scattered throughout the nucleus like a fine dust; the delicate threads of the later stages being formed at the expense of this dust-like substance.

In the germinal vesicles of *Euschistus variolarius* the chromosomes first appear as extremely fine threads, which seem to arise as a concentration of the distributed granular mass, these granules gradually disappearing as the threads become more defined. Photo 17 shows a germinal vesicle at the stage just before the chromosomes appear — the chromatin is still distributed as granules throughout the nucleus, although the size of this nucleus is nearly equal to many of the germinal vesicles in which the chromatic threads are fully developed. In Photos 18 and 20 we see the delicate chromatic threads just appearing, as yet very indistinct, and in the later stage shown in Photo 21 the threads are more distinct but too much tangled to warrant any precise interpretation. In later stages, however, we can often trace among the filaments, an unbroken thread, so long that it cannot possibly be interpreted as one of the fourteen univalents, it must represent at least two univalents attached end to end. Again some of these threads are long enough to justify the inference that they represent even more than a bivalent, indicating that in *Euschistus* the chromosomes emerge from the rest stage not as univalents, but as long threads representing at least one or more bivalents, thus supporting the observations of those who believe that bival-

ents arise by the omission of a transverse division of a spireme, rather than by a conjugation of univalents. We believe that the evidence in *Euschistus* is opposed to the interpretation that the chromosomes first appear as univalents and later conjugate, either longitudinally or end to end. We believe, rather, that there is a tendency to form a continuous spireme, because we find cases in which very long, thin, unbroken threads are present, and a few examples of this kind outweigh as evidence, a large number of cases where we find the threads broken into small pieces. These delicate threads could easily be broken by the technique, for after pricking, the germinal vesicles flatten and dry on the slide, and this disturbance, combined with the shrinkage of drying, could easily account for the rarity of the cases in which the long thin threads remain unbroken.

In Photo 24 we see this tendency to the formation of a spireme — a few of the threads can be traced certainly too far to justify any assumption that they are univalent or even bivalent chromosomes. A long unbroken thread is also demonstrated in Photos 23, 28 and 29.

Tellyesniczky ('07) claims that the long thin chromatic threads do not become shorter and thicker by contraction. He thinks rather this is accomplished by a flowing together and change of place of the substance of which the thread is composed. He says, there is a continual disintegration and rebuilding of the threads and the new structure is formed at the expense of the old.

We believe this is not the case in *Euschistus*. For example, it seems reasonable to interpret the chromosomes of Photo 30 as due to contraction of long thin bivalents (as shown in Photo 25), rather than to regard them as entirely new formations. Such a detail as this is interesting, but it seems to us only incidental to the all-important question, do the chromosomes retain their individuality throughout the rest stages — do they differ in this particular from other structures of the cell — the centrosome — the aster — the spindle — the nucleolus, etc. These elements return at each cell generation with as much regularity as to size and form as do the chromosomes, but those who believe in the individuality and continuity of the chromosomes attribute to the latter structures a causal significance not now ascribed to any other organ of the cell.

In the past, however, similar claims have been made for the centrosome — its individuality and continuity were asserted by many cytologists, some going so far as to believe the centrosome to be the cause rather than the expression of cell activity.

Certain facts indicate that all the chromatic substance of the germinal vesicle of *Euschistus* is not used for the chromosomes. We find in many germinal vesicles various segregations of chromatic substances which are present after the chromosome threads are formed and which often do not entirely disappear until the prophase stage. We sometimes find irregular granular or homogeneous masses as in Photos 20 and 22, sometimes numerous large granules, so dense that they look like very small nucleoli (Photos 19, 21, 23, 26, 27 and 29), and sometimes merely deeply staining areas, as shown in Photos 23, 24 and 27. We have no means of proving that this chromatic substance, not used for the chromosomes, is chromatin, but for many forms it has been claimed that only a small part of the chromatin is used in the formation of the chromosomes and we are therefore perhaps justified in surmising that the chromatic substance in *Euschistus* (which often persists until after the chromosomes are formed) may be chromatin residue, which assumes various forms during its disintegration and disappearance.

The early stages of the development of the germinal vesicles shown in Photos 11 to 17 we interpret as indicating that the chromosomes completely disintegrate — that any claim of their morphological persistence during these stages must be made as a pure assumption, as there is no morphological evidence whatever of their persistence. On the contrary there is every indication that they completely disintegrate. We believe we have no reason to assume that the reappearance of the chromosomes at certain stages differs essentially from the reappearance of the centrosome, aster, spindle, nucleolus and other cell structures.

Fick ('07) in his critical review of the inconclusive evidence that has been offered as proof of the individuality and continuity of the chromosomes, and of their causal character, sounds a welcome note of protest against recent chromosome speculations. In addition to Fick such experienced cytologists as Häcker and Meves, have expressed their skepticism of the sex-

determination hypothesis in no uncertain terms, and many of the investigators who have studied the accessory chromosomes in insects are equally incredulous, Montgomery, Schäfer, Moore, Robinson, Arnold and others.

We have reserved a description of the later stage of the germinal vesicle chromosomes of *Euschistus* for a subsequent publication, which will discuss only the chromosome groups in both male and female cells. In the male cells we have traced the idiochromosomes through both maturation divisions and are able to support, in the main, Wilson's results as to their division in these stages. He says, the idiochromosomes remain univalent in the first division, each dividing independently, and in the second division the two separate — the small idiochromosome going to one pole and the large idiochromosome to the opposite pole. He gives no details however as to the method of the first division and as we find an interesting irregularity in this division, we believe it is worthy of consideration. As the idiochromosomes separate in the second spindle, this is presumably the so-called reduction division for this unequal tetrad, and the first division should be therefore an equation division, and *both* idiochromosomes should divide longitudinally, if the significance that has been attributed to the longitudinal and transverse divisions of the chromosomes holds true in this form.

The value of the significance of a longitudinal or transverse division is based on the assumption that the so-called ids are arranged in a row, and a morphological demonstration of this assumption is claimed for those cases in which the chromosomes are rod- or thread-shaped — these rods or threads often appearing as a single row of chromomeres.

In our smear preparations of the testes of *Euschistus variolarius* both the large and small idiochromosomes are distinctly rod-shaped, and at the late first prophase and metaphase it can be clearly demonstrated that the *large rod divides longitudinally and the small rod transversely*. There are rare (perhaps only apparent) exceptions to this, but as a rule one divides transversely *just as surely and as constantly* as the other divides longitudinally, and this we have demonstrated in a number of photographs.

The germ cells are being studied in order to obtain a morpho-

logical basis for conclusions or to put conclusions to a morphological test, and the idiochromosomes seem to offer a fruitful test of the theoretical value of longitudinal and transverse divisions. In order to retain our faith in the special significance that has been attributed to the longitudinal and transverse divisions we are driven to the inconsistency of accepting the evidence in the case of one idiochromosome, and disclaiming it for the other — though the evidence in both cases is equally strong. If, on the other hand, we accept the evidence and at the same time retain faith in the theory, we are forced to admit that the sister cells of the first division are also dimorphic and we have a so-called reduction division of one of the idiochromosomes of the first spindle as surely as we have a reduction division (the separation of the two idiochromosomes) in the second spindle, we thus have a somewhat similar phenomenon occurring in both spindles.

We have a number of photographs demonstrating both spermatocyte divisions and also a large number showing the spermatogonial, oögonial and embryonic chromosome groups — several hundred in all, but the publication of these must be reserved for a separate paper, where we shall aim to make a very full comparison of these chromosomes, giving especial attention to the groups that have not been presented in the papers which advocate the sex-determination hypothesis. We believe that these groups are of special value — that we have no right to ignore exceptions because they do not fall in line with certain theories. The trite claim that exceptions prove the rule loses its force when enough exceptions are found to challenge the rule. A careful examintion of our preparations makes it possible to select chromosome groups which exactly fit a given theory, but many groups can also be found that are a serious menace to these theories, while on the other hand they present no difficulties to the conception of those who regard the number, size and form of the chromosomes as inherited characters — the expression of cell activities rather than the cause.

January, 1909.

Appendix.

We have just received Wilson's latest "Study on Chromosomes," No. V.,[1] in which (in a footnote on page 159) he attempts to explain what he calls our "entirely mistaken conclusions" regarding the division of the accessory chromosome in *Anasa tristis* as follows:

"The regrouping of the chromosomes in the second division, first described by Paulmier ('99) in *Anasa tristis*, is characteristic of the Coreidæ generally, an eccentric position of the idiochromosome being a nearly constant feature of the first division but not of the second. Failure to recognize this fact in the case of *Anasa tristis* seems to have been one of the main sources of error in the entirely mistaken conclusions of Foot and Strobell ('07*a*, '07*b*) regarding this species. (Cf. Lefevre and McGill, '08.) Demonstrative evidence on this point is given by polar views of rather late anaphases, in which every chromosome of each daughter plate may be seen, in the same section. Such views, of which I have studied many, both in *Anasa* and other genera, show that one of the chromosomes may indeed occupy an eccentric position, and may there divide; but in such cases the odd chromosome is always found elsewhere in the group, lying either in or near one of the daughter groups and not in the other. When the odd chromosome is eccentric it is found in one of the daughter groups but not in the other."

In order to speak with authority on the regrouping of the chromosomes, some accuracy at least is expected in the identification of the individual chromosomes. Paulmier's authority can be dismissed when we recall Wilson's interesting discovery that in *Anasa tristis* Paulmier erroneously identified the microchromosome as the accessory (undivided) chromosome of the second spindle.

In Plate III. of our "Study of Chromosomes in the Spermatogenesis of *Anasa tristis*" ('07) we *show* in Photos 27, 28, 33, 35, 36, 37, 38, 39, 42, 43, 44 and 45, late anaphases or early telophases of second spindles demonstrating the ring-like formation of the chromosomes, with one of the chromosomes eccentrically placed or lagging. In our smear preparations this grouping is

characteristic of the second division as it is of the first, although, as we have already pointed out, there are exceptions to this rule. There can be no possible doubt of our identification of these groups as second spindles, because they are invariably found in regions of the testis surrounded by spermatids in different stages of development and, further, the form of the chromosomes is so beautifully demonstrated in our smear preparations, it is hardly possible to confound the first and second spindles,[1] a mistake easily made in sections. That the individual chromosomes may change their relative positions at this stage, while the form of the group remains unchanged, is a possibility that any observer would naturally consider. But as we find in our preparations, the ring-like grouping of the chromosomes of the first spindle so constantly duplicated in the second spindle, with the micro-chromosome (the one chromosome that can be identified beyond question) maintaining its characteristic position in the center of the ring, the identification of the eccentric chromosome of both spindles as *probably* the same chromosome, is certainly as legitimate a conclusion, as forcing an arbitrary contradiction of this assumption, in order to support a theory.

In complete opposition to what Wilson calls his "demonstrative evidence" on this point, we refer to our published photographs, Plate III. (in the paper quoted above), 29, 32, 33, 34, 35, 36, 37, 40, 41, 43, showing polar views of late anaphases or telophases of second spindles, in which every chromosome of each daughter plate can be counted. In all these preparations there is an eccentric, lagging — in most cases dyad — chromosome, but as *ten* chromosomes can be clearly counted at each pole, we fail to see how the situation is improved, for Wilson to claim, that where the lagging chromosome divides in this spindle it is not to be identified with the "odd" chromosome — which he says, "is, in such cases, *always to be found elsewhere in the group.*" We have given demonstrative evidence that this is not so in our preparations.

The essential fact is not the relative position of the accessory chromosome in either the first or second spindles, but whether

[1] The ring-like grouping of the chromosomes is also characteristic of the first and second spindles of *Euschistus variolarius*, where the distribution of the unequal tetrad in the second spindle adds conclusive proof of the identity of the spindles.

one of the chromosomes consistently fails to divide in the second spindle. It has been said of the accessory chromosome that in the spindle in which it is heterotropic, it tries to divide, but does not succeed. In the case of *Anasa tristis*, we have demonstrated that it is by no means always unsuccessful in these efforts.

We regret that we cannot agree with Professor Wilson as to the value of the support he believes he has received from Lefevre and McGill's paper on *Anasa tristis* and *Anax junius* ('08). In this paper our results in *Anasa* are summarily disposed of in two pages of curt contradictions, supported by ten sketches made from sections. The futility of such methods as opposed to the very full demonstration we have given in 107 photographs to support our conclusions in *Anasa*, must be apparent to any unprejudiced observer.

In this same paper, McGill corrects her earlier observations on *Anax junius* on three very important points :

1. In her first work on this form ('04) (in which she expresses her indebtedness to Professor Lefevre) she found no evidence of a chromatin nucleolus in the resting spermatocyte. She and Lefevre now find, *in the same material*, a chromatin nucleolus persisting through the resting spermatocyte.

2. In her earlier work McGill identified the microchromosome as the accessory chromosome. She and Lefevre now identify, *in the same material*, one of the larger spermatogonial chromosomes as the accessory chromosome.

3. In her original count of the spermatogonial chromosomes, McGill found an even number, 28. She and Lefevre now find *in the same material* an odd number, 27 spermatogonial chromosomes.

In view of these contradictions we may justly hesitate to accept as definitive the recent conclusions reached by McGill and Lefevre in *Anasa tristis*, believing a new point of view may give us still further variations in their very interesting observations.

BIBLIOGRAPHY.

Arnold, George.

'08 The Nucleolus and Microchromosomes in the Spermatogenesis of Hydrophilus piceus (Linn.). Arch. f. Zellforschung, Bd. II., Hft. I.

Deton, Willy.

'08 L'étape Synaptique dans l'ovogénèse du Thysanozoon Brocchii. La Cellule, t. XXV.

Fick, R.

'07 Vererbungsfragen, Reduktions und Chromosomen-hypothesen. Ergebnisse der Anat. u. Entwick-geschichte, Bd. XVI., 1906.

Foot and Strobell.

'05 Prophases and Metaphase of the first Maturation Spindle of Allolobophora fœtida. Amer. Jour. Anat., Vol. IV., No. 2.

'07 A Study of Chromosomes in the Spermatogenesis of Anasa tristis. Amer. Jour. Anat., Vol. VII., No. 2.

Häcker, Valentin.

'07 Die Chromosomen als angenommene Vererbungsträger. Ergebnisse u. Fortschritte der Zoologie, Bd. I., Hft. I.

McClung, C. E.

'02 The Accessory Chromosome — Sex Determinant? Biol. Bull., Vol. III., No. 2.

Meves, Fr.

'02 Struktur und Histogenese der Spermien. Ergeb. der Anat. u. Entw.-geschichte, Bd. XI., 1901.

'08 Es gibt keine parallele Konjugation den Chromosomen. Arch. f. Zellf., Bd. I., Heft. 4.

Montgomery, Th. H.

'98 The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., Bd. XII.

'01 A Study of the Chromosomes of the Germ Cells of Metazoa. Trans. Amer. Philos. Soc., Vol. XX.

'06 Chromosomes in the Spermatogenesis of the Hemiptera heteroptera. Trans. Amer. Philos. Soc., Vol. XXI.

Moore, J. E. S., and Robinson, L. E.

'05 On the Behavior of the Nucleolus in the Spermatogenesis of Periplanata Americana. Quart. Journ. Mic. Sci., Vol. XLVIII.

Schäfer, Friedrich.

'07 Spermatogenese von Dytiscus. Zool. Jahrb., Bd. XXIII., Hft. 4.

Stevens, N. M.

'05–'06 Studies in Spermatogenesis. Publication No. 36, Parts I. and II., Carnegie Institution of Washington.

v. Tellyesniczky, Koloman.

'07 Die Entstehung der Chromosomen. Evolution oder Epigenese? Urban & Schwarzenberg, Berlin.

'05 Ruhekern und Mitose. Arch. f. mik. Anat., Bd. 66.

Wassilieff, A.

'07 Die Spermatogenese Von Blatta germanica. Arch. f. mik. Anat., Bd. 70.

Wheeler, William Morton.

'97 The Maturation, Fecundation and Early Cleavage of Mysostoma glabrum Leuckart. Archive de Biologie, Tome XV.

Wilson, E. B.

'05 Studies on Chromosomes, I. Journ. Exper. Zoöl., Vol. II., No. 2.

'05 Studies on Chromosomes, II. Journ. Exper. Zoöl., Vol. II., No. 4.

Explanation of Plates.

All the photographs were taken with a Zeiss Apo. 2 mm. immers. lens, 140 apr. and compensating ocular 4. Photos 1 to 31 are from smear preparations stained with a saturate solution of Bismarck brown. In all these preparations the *whole nucleus* is shown.

Magnification of Photos 1 to 10 inclusive, 1,000 diameters.

Magnification of Photos 11 to 32 inclusive, 600 diameters.

As some of the germinal vesicles were too large to be included in the field at a magnification of 1,000 diameters, we used a magnification of 600 diameters for all the oöcytes, in order to facilitate a comparison.

The reproductions were made by the Rotograph Company from our own negatives.

Plate I.

Euschistus variolarius.

Photo 1. Two young resting spermatocytes, first order, one showing two nucleoli and the other one nucleolus.

Photo 2. A little later stage than Photo 1. Two nucleoli are shown, unequal in size, but this inequality is not so marked as in the two nucleoli of Photo 6.

Photo 3. Resting first spermatocyte about the same stage of development as Photo 2. One nucleolus present.

Photo 4. A resting first spermatocyte showing two nucleoli about equal in size.

Photo 5. A resting first spermatocyte with two nucleoli, unequal in size, though the inequality here is not so conspicuous as in Photo 6.

Photo 6. A later stage than Photo 5. The chromatin shows the segregation which precedes the formation of the chromosomes. Two nucleoli are present, very unequal in size.

Photo 7. First spermatocyte with the chromatin somewhat more closely segregated than in Photo 6. One nucleolus is present showing a typical vacuole.

Photo 8. A larger first spermatocyte with one nucleolus, the disposition of the chromatin suggesting a network.

Photo 9. A first spermatocyte showing an early stage in the formation of the chromosomes; one nucleolus is present.

Photo 10. A much later stage than Photo 9. The bivalent chromosomes are formed, but the number is not so clearly demonstrated as at later stages. (These later stages, as well as the transitional stages, between Photos 9 and 10, are reserved for a subsequent publication.) The nucleolar-like thickening in the loop of the chromosome on the lower periphery of the group is not a small nucleolus. In the preparation it can be plainly recognized as a segregation of chromatin at the point where a loop of the chromosome brings two parts of the thread in contact. Similar, though less conspicuous thickenings are seen at other points where two threads cross. One clear nucleolus is present in this spermatocyte.

Photo 11. Nucleus of a young oöcyte, with the chromatin granules quite evenly distributed throughout the nucleus. No nucleolus present.

Photo 12. A slightly older germinal vesicle, with the chromatin segregated into clumps, too numerous to represent individuual chromosomes.

Euschistus variolarius. PLATE I

FOOT & STROBELL PHOTOS.

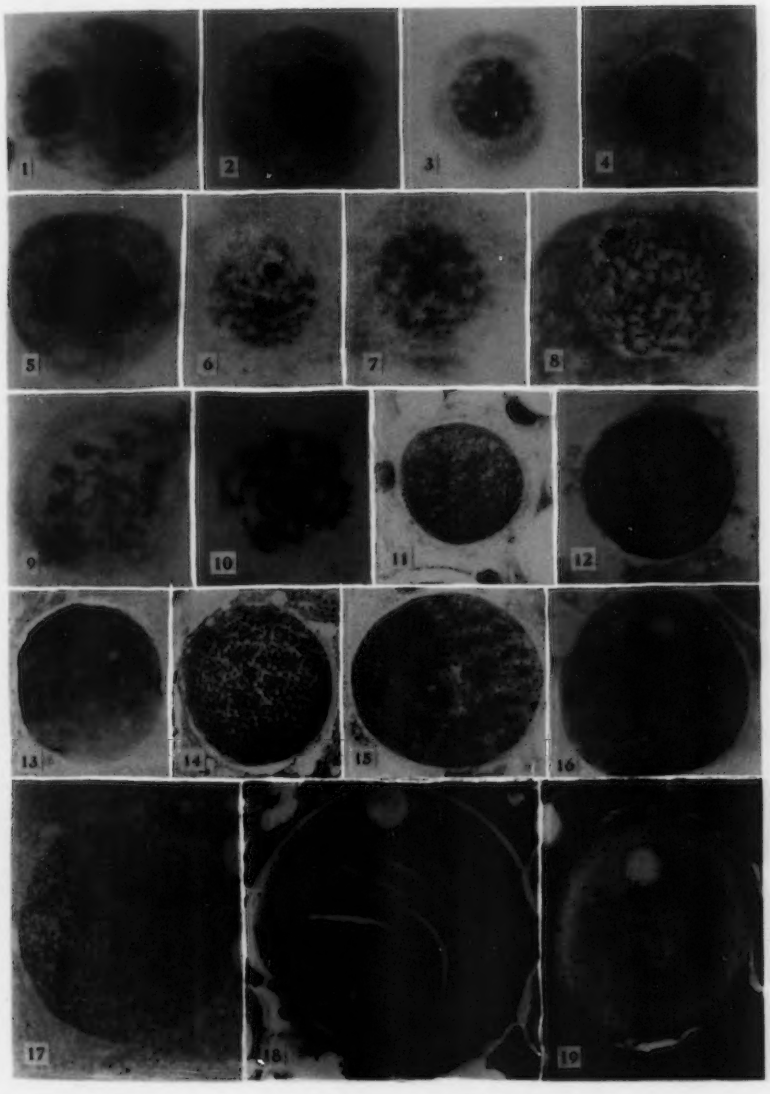


PHOTO 13. A young germinal vesicle with the chromatin almost in a diffused condition. A small, clear, spherical space is present, which may represent the first appearance of the achromatic nucleolus so conspicuous in the older germinal vesicles of Photos 18 to 30.

PHOTO 14. A young germinal vesicle with the chromatin evenly distributed as in Photo 11.

PHOTO 15. An older germinal vesicle with the chromatin segregated into numerous clumps as in Photo 12. Many of the clumps in this germinal vesicle appear almost as dense as nucleoli, though in the preparation they are seen to be merely a close segregation of fine granules.

PHOTO 16. An older germinal vesicle showing less pronounced segregations of chromatin, and a clear spherical space which can surely be interpreted as an early appearance of the achromatic nucleolus seen in the germinal vesicles of Photos 18 to 30.

PHOTO 17. A much larger germinal vesicle with the chromatin quite evenly distributed as granules.

PHOTO 18. An older germinal vesicle with the first indication of the formation of delicate chromatic threads, which later develop into the chromosomes. At this stage the achromatic nucleolus is always present. The cracked spaces shown in this germinal vesicle and in Photos 21, 23, 24 and 29 are due to uneven shrinkage as the germinal vesicles rapidly dry on the slide.

PHOTO 19. A much later stage than Photo 18 — the chromatin threads being well formed. (The next stage to Photo 18 is shown in Photo 20, Plate II.) The germinal vesicle of this preparation is not well flattened and some of the chromatic threads are very much out of focus.

PLATE II.

Euschistus variolarius.

PHOTO 20. A germinal vesicle a little further developed than the one shown in Photo 18. The delicate chromatic threads are a little more defined. The achromatic nucleolus is present near the periphery.

PHOTO 21. A germinal vesicle with the delicate chromatic threads well formed. The achromatic nucleolus is seen on the periphery.

PHOTO 22. A germinal vesicle with the chromosome threads further developed. The nucleolus near periphery.

PHOTO 23. A germinal vesicle with long, thin chromosome threads. The nucleolus on periphery.

PHOTO 24. A germinal vesicle at about the same stage of development as Photo 23 The nucleolus on periphery.

PHOTO 25. A germinal vesicle with the chromosome threads further developed.

Euschistus variolarius.

FOOT & STROBELL PHOTOS.

PLATE II

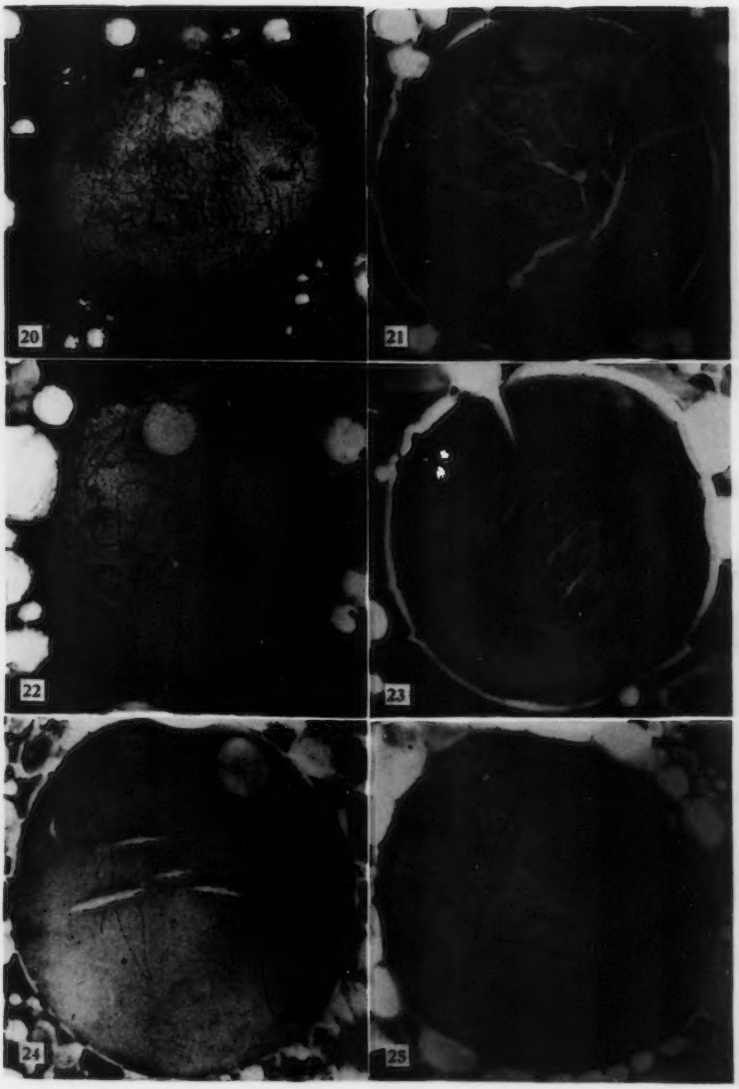


PLATE III.

Euschistus variolarius and Allolobophora fœtida.

PHOTO 26. A germinal vesicle with chromosome threads well formed. Nucleolus on periphery.

PHOTO 27. A germinal vesicle at about the same stage of development as Photo 26. The nucleolus near the center.

PHOTO 28. A germinal vesicle with long, thin, twisted chromosome threads. Nucleolus near the center.

PHOTO 29. A germinal vesicle showing a later stage of development. The chromosome threads have contracted into shorter, thicker pieces. Nucleolus still present.

PHOTO 30. A germinal vesicle with the chromosomes almost at first prophase stage. Nucleolus still persisting.

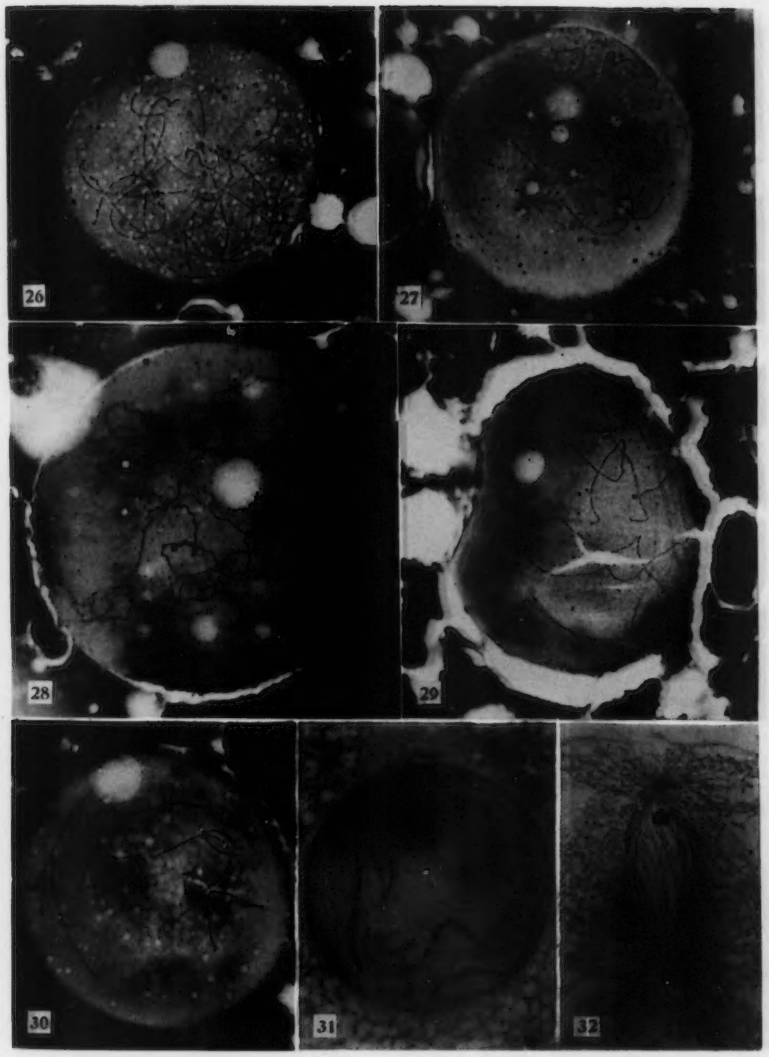
PHOTO 31. A germinal vesicle of *Allolobophora fœtida*. The same technique as that used for the *Euschistus* germinal vesicles (Photos 18 to 30). The chromosome threads are long, and thin, and longitudinally split. The large, less chromatic nucleolus is on the periphery, and the dense chromatin nucleolus is near the center.

PHOTO 32. A section (2½ μ thick) through the first maturation spindle of *Allolobophora fœtida*. At the equator one chromosome is shown, the ten remaining chromosomes being clearly demonstrated in the adjoining sections. Near the upper pole we see the persisting chromatin nucleolus. A centrosome is shown at each pole

Euschistus variolarius.

PLATE III

FOOT & STRODELL PHOTOS



INHERITANCE IN THE "WALKING-STICK," APLOPUS MAYERI.

CHARLES R. STOCKARD.

The family Phasmatidæ, as is well known, shows some of the most striking cases of "protective resemblance" found among the insects. Several of the genera are typically stick-like to a surprising degree while members of the genus *Phyllium* resemble in detail a leaf-like structure. These animals are no doubt protected by their imitative forms provided they behave in a certain manner. In fact the protection or concealment of such an animal depends as largely on its behavior as upon its resemblance to surrounding objects. In order to ascertain whether these so-called protectively adapted insects really exhibited a "protective behavior" I[1] studied the habits of the "walking-stick," *Aplopus mayeri*, which is abundant on the Tortugas Islands, Florida. These large insects were found to behave in a manner almost ideal for their concealment among the twigs and stems of the plant on which they feed, *Suriana maritima*.

My study was made during a season, June and July, when the enemies of *Aplopus* were extremely rare on these islands. In the spring and fall, however, the great numbers of migrating birds which stop here no doubt devour many of these large Orthoptera in spite of their almost perfect concealment. But for their protective resemblance and habits birds might easily exterminate such slow-moving flightless insects within a few seasons, in fact the existence of creatures like *Aplopi* on these small islands is really dependent upon their ability to be passed unobserved by birds migrating between the eastern United States, West Indies and South America.

The question arises whether the protective behavior in *Aplopus* is fully developed on hatching from the egg or whether it is attained with their large size and mature condition. In order to

[1] "Habits, Reactions and Mating Instincts of the 'Walking-Stick,' *Aplopus mayeri*," *Science*, N. S., XXVII., 1908—Publication No. 103, Carnegie Institution, Washington, 1908.

investigate this and the further question of first-leg form considered below eggs were collected during the summer and brought to New York where the newly hatched individuals might be observed.

These eggs were kept in small loosely stoppered bottles in the laboratory at ordinary room temperature. They began hatching about the first of December and during January a large number of the insects came out.

Behavior of Newly Hatched Aplopi.

The reactions of the small insect on the day it emerges from the egg are almost identical with those of the fully mature eight-inch females which I studied at the Tortugas. The young *Aplopi* are a light chocolate-brown in color with yellowish bands about the legs and the sexes are similar. The adult males, however, become greenish in color while the female retains her original brown. The adults also have rudimentary wings which are capable of being raised when the insect is excited, but the young are wingless. Their reactions will be referred to briefly at this time as they are given in some detail in my former paper.

The insects when at rest among the twigs assume an attitude which in consequence of their stick-like shape makes them most difficult to detect. The first pair of legs are stretched directly forward enclosing the head between thin curved portions of the femora which fit perfectly against it. The antennæ are brought

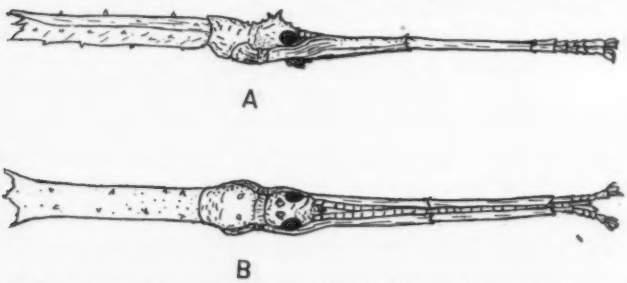


Fig. 1. *A*. Lateral aspect of *Aplopus*, head and first two thoracic segments. The first pair of legs are stretched forward in the typical resting attitude. The femur fits perfectly about the ventro-lateral region of the head and leaves the eyes uncovered.

B. Dorsal view of the same specimen showing the approximated antennæ directed forward and enclosed between the first pair of legs.

together between the first legs, Fig. 1, *B*. (See also Fig. 1, Plate I., and the photographs in my former paper.) The anterior end of the insect thus closely resembles a more or less pointed stick. The newly hatched individual habitually assumes this position and the point of especial interest, which I shall return to later, is that the thinned out curved part of the femora fit about the head as perfectly the first time the legs are stretched forward as they do in the adult.

In walking from place to place the adult moves slowly and often exhibits a slow laterally swinging motion suggesting a twig swinging in a light breeze. The young also swings its body from side to side in a similar manner. When a number of young *Aplopi* are sitting motionless if the observer blows a current of air over them they all begin to swing very actively from side to side as if being swung by the breeze. This swinging motion no doubt serves to render them less conspicuous among the shrubs.

The newly hatched individuals use the same methods to escape an enemy as those employed by the adult. When they are touched or pinched slightly they move away a short distance and immediately come to rest again, if the stimulus be repeated they begin to walk at a more rapid gait than before and move a greater distance away. If again touched they drop bodily to the floor and feign death just as the adult does. The death-feigning reaction is more readily induced in the young than in the adult, and no doubt serves to great advantage in enabling them to escape an enemy which fails to seize them securely in the first attempt. The chances of escape for this stick-like creature when it drops through the dense foliage and branches of the *Suriana* bush is most favorable. When in the death-feint the legs may be bent in any position and the body twisted without the least move on the part of the animal. They may actually be piled one on another and will remain as motionless as dead insects.

The young walking-stick crawls upwards on any object that it may reach after emerging from the egg. As I previously recorded the female *Aplopus* sits in the *Suriana* bushes and lays its eggs which fall to the ground where they later hatch. Thus the tendency of the young to crawl upwards on the first object with which it comes in contact serves to bring it up the *Suriana* bush

to its leafy food. In crawling up the young insect waves its antennæ to feel the way just as does the adult and reaches out with the first legs to grasp the object located by the antennæ.

Finally the young like the adult is more or less nocturnal in its movements. During the day they sit motionless with the first legs extended forward but at night they become active and move about to feed. The food of the adult is limited to the leaves of *Suriana*. I have made no attempt to feed the young since they may be kept alive for about one week after hatching without taking food.

The Thin Curve of the Femora which Fits Against the Ventro-Lateral Surfaces of the Head.

When the first pair of legs are extended forward the femur of each is so curved near its proximal joint as to fit perfectly against the ventro-lateral parts of the head and at the same time leaves the eyes uncovered, Fig. 1, *A* and *B*. The curved portion of the femur is also very thin in a lateral direction and thus when pressed closely to the head the legs go out as almost straight lines instead of bulging around the head to any great extent. It seems difficult to believe that the first pair of legs could through chance variations or mutations have come to fit so perfectly around the sides of the head and at the same time to have their dorsal line so curved as to leave the eyes uncovered. It must be remembered that when the first legs are in the extended position the head presses against the dorso-lateral surfaces of the femur and not straight against the inner lateral surface only. This arrangement may be better understood by a close examination of the dorsal and lateral views given in Fig. 1, *A* and *B*.

The possibility suggests itself that the perfection of the fit is attained during the life of the individual since the legs are so habitually pressed against the head for about twelve hours daily. To test this it became desirable to study newly hatched individuals in order to find whether the femur curve was as perfectly adjusted in them as in the adult. A careful examination of about one hundred *Aplopi* shortly after emerging from the egg has convinced me that the curve of the femur is as true to the head pattern in the newly hatched young as in the mature insect when several months old.

Finally, is this adjustment between the structure of the femora and head due to the position of the insect when enclosed within the inelastic egg shell? If the first legs were folded forward against the head the pressure during embryonic life might easily be sufficient to mold the femur curve into pattern for the head. Twenty eggs containing embryos at various stages shortly preceding hatching were dissected with this question in view. The elliptical egg shell is of a rigid chitinous material with a circular operculum at one end and a hilum-like scar on one side to which the

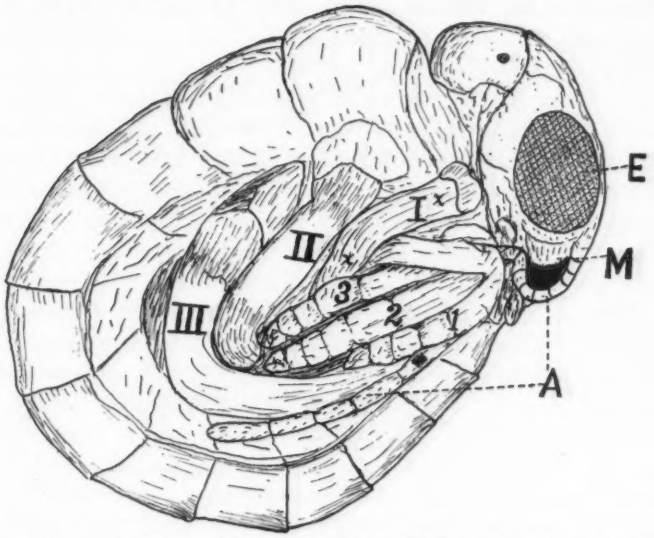


FIG. 2. An unhatched *Aplopus* with its egg membranes dissected away showing its folded position. The femora of the three right legs are marked with roman numerals and the tarsal ends of the same legs are indicated by figures. The part of the first femur which in later life fits against the head is shown between the points *x*–*x*, it is not molded against the head in the egg. *A*, antennæ; *E*, compound eye; *M*, mandible.

inner egg membrane is attached. The size of the egg ranges from 2 mm. in narrow diameter by 4 mm. in long diameter to 3 mm. by 4.5 mm. The length of the walking-stick on hatching is from 17 mm. to 23 mm. measured from the tip of abdomen to tip of the first pair of legs when extended forward, or from tip of head to tip of tail 9.5 mm. to 13 mm. The embryo within the egg is, therefore, necessarily much folded and bent.

On dissecting the egg the embryo is found to be curled around in a rather constant manner, the head being usually, though not always, near the opercular end. The long legs are folded back and forth upon themselves in a very definite fashion as shown by the camera drawing, Fig. 2. The antennæ (*A*) pass down the front of the head and then back along the ventro-lateral surface of the abdomen being sometimes bent around the first pair of legs. The point of most importance is that the femoral segments of the legs are all directed obliquely away from the head. The first pair of legs each of which is folded on itself four times does not touch the sides of the head at all. The head and large eyes are entirely uncovered and exposed. The femora of the first pair of legs not only fail to mold their curves against the head but the femora are so pressed against the thorax that the surfaces which will subsequently be concave (in Fig. 2 between *x* and *x*) are actually arched convexly. Thus it is seen that the mechanical arrangement of the embryo's parts within the egg is not responsible for the fit of the femur curve against the head. On the contrary the curve seems to develop in spite of these arrangements.

When hatching the embryo's head and body come forth from the egg first, the antennæ are then pulled out, the legs being the last parts liberated from the shell, Fig. 3. It often happens that the shell is carried around for some time dangling to the third pair of legs. In Fig. 3 the well developed curves of the femora are distinctly shown, *x* to *x*, and are being pulled in a direction away from the head, yet as soon as the legs are free from the shell the first pair may be straightened forward and their curved femora fit neatly against the sides of the head. We see, therefore, that the curve of the femora to fit the sides of the head is a character transmitted to all of the young and perfectly formed at the time of hatching. It might seem that the origin of this character was most probably due to the habit of the insects to press the first pair of legs against the head. Gradually this pressure developed a thin concave region of the femur of the first leg which molded itself more and more perfectly to the contour of the head. If this curve arose in any other way the second and third pairs of legs might have developed at least a trace of such a character though this is not absolutely necessary. It must be

remembered that the curves fit the ventro-lateral contour of the head to a remarkable degree.

When the first pair of legs are so stretched forward the insect's antennæ are brought together. The legs have an irregular groove extending along the approximated surfaces and when complete approximation takes place a rather imperfect tube is formed enclosing the antennæ. This is a case analogous to the above and it is difficult to imagine how chance variations could

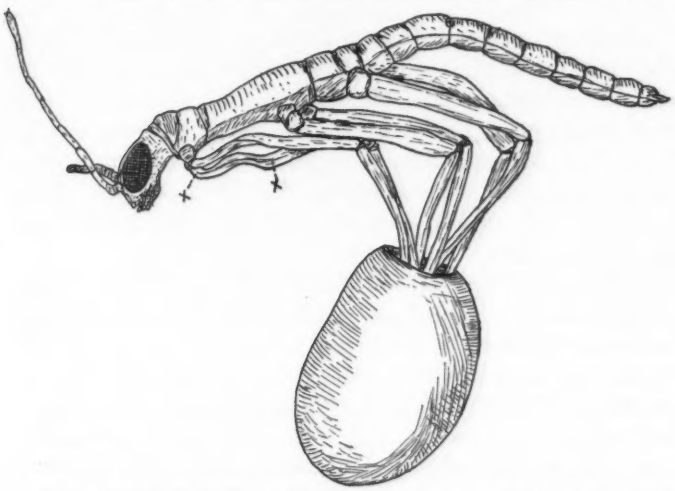


FIG. 3. *Aplopus* in the act of hatching from the egg. The body and head come out first, then the antennæ and finally the legs free themselves from the shell. The parts of the first legs between *x*–*x* are curved to fit the head when they are straightened forward although they have never touched the head up to this time.

bring about such mechanical harmonies between organs only associated through an habitual attitude assumed by the animal when at rest. Yet it must not be forgotten that many other equally as nice morphological arrangements exist which have no habit or action connected with them. Indeed a crucial case of use inheritance is almost impossible to imagine from purely descriptive work. I would not be understood as advocating any principle of inheritance but merely bring forward the present case as being of interest in itself.

CORNELL MEDICAL SCHOOL, NEW YORK CITY,
February 6, 1909.

THE CONNECTIONS OF THE GONADIAL BLOOD VESSELS AND THE FORM OF THE NEPHRIDIA IN THE ARENICOLIDÆ.

ELLIOT ROWLAND DOWNING, PH.D.,
NORTHERN STATE NORMAL SCHOOL, MARQUETTE, MICHIGAN.

The gonads of *Arenicola* occur on certain blood vessels that lie diagonally on the exterior of the glandular portion of the nephridia and that are consequently designated the gonadial vessels. In a study of the gonads, the results of which will be published shortly, it was found that the literature of the subject contained conflicting and inaccurate statements regarding the relations of these gonadial vessels to their connecting vessels. This paper is the result of an attempt to determine these relations.

Gamble and Ashworth, in 1900, published an extended study of the anatomy of the several species which also reviewed the important literature on the family. They had previously published, 1898, a paper on the anatomy of *Arenicola marina*. In an article published in 1899, V. Willem disagreed with some of their statements as did R. Lillie in a more recent publication, 1906. These contradictory statements I shall try to adjust: I must also take exception to some other statements of each.

The general relations of the blood vessels may be easily understood from the accompanying partly diagrammatic figure of a cross-section at about the level of the first nephridium of *A. cristata* (Fig. 1). It will be noted that there are two main blood vessels, a dorsal and a ventral; these run the entire length of the animal. There is a pair of neurals of much smaller caliber, also extending the full length of the body. The paired gastric laterals and subintestinals, as also the paired integumentary vessels known as the parietal (or dorsal longitudinal) and the longitudinal nephridial, are limited in their extent and vary in length and prominence in the several species.

All previous authors agree in the arrangement of the nephridial vessels as follows: The afferent vessel, on approaching the

nephridium, divides, one branch going to the setal sac and gill, if present, one to the integumentary vessels and one to the nephridium. The latter enters the nephrostome and forks, one branch traversing each lip. After reuniting, the vessel, reformed, runs out onto the wall of the nephridium as the gonadial vessel. Each of the three main branches of the afferent vessel gives off smaller vessels which, with more or less anastomosing with adjacent vessels, form capillary net-works in the organs supplied.

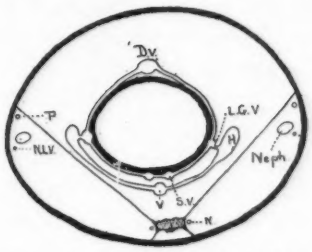


FIG. 1. Partly diagrammatic cross-section of *Arenicola cristata* at about the level of the first nephridium. *D.V.*, dorsal blood vessel. *H.*, heart. *L.G.V.*, lateral gastric blood vessel. *N.*, neural blood vessel. *N.L.V.*, nephridial longitudinal blood vessel. *P.*, parietal blood vessel. *S.V.*, sub-intestinal blood vessel. *V.*, ventral blood vessel.

The efferent vessels are formed by the union of capillaries in these same regions.

The afferent vessel, as a rule, comes from the ventral vessel. This is true for all except the first two nephridia of *A. Grubii* and of *A. ecaudata*, which nephridia are supplied, the first by a branch of the dorsal, the second by a branch of the parietal vessel (Fig. 2, *d*). In addition to the afferent vessel from the ventral vessel, the following nephridia also receive branches from the dorsal vessel: the first of *A. cristata*, the first two of *A. Claparedii* and the first three of *A. marina* (Fig. 2).

The following nephridia return blood directly to the subintestinal vessels through efferent vessels whose numerous branches are adjacent to their funnels: the fourth, fifth and sixth of *A. cristata*, the fourth and fifth of *A. Claparedii* and the fifth and sixth of *A. marina*. All others must pour the blood into the parietal and nephridial longitudinal vessels which, in turn, pass it to some of the more posterior efferent vessels (Fig. 2).

These observations are directly antagonistic to those of other investigators on several points of anatomical detail and of function of some of the vessels. I have given above a statement of the general arrangement of the branching afferent vessels with reference to the nephridia; it will be evident from text-figure 2 that my results only agree in a general way with the statements

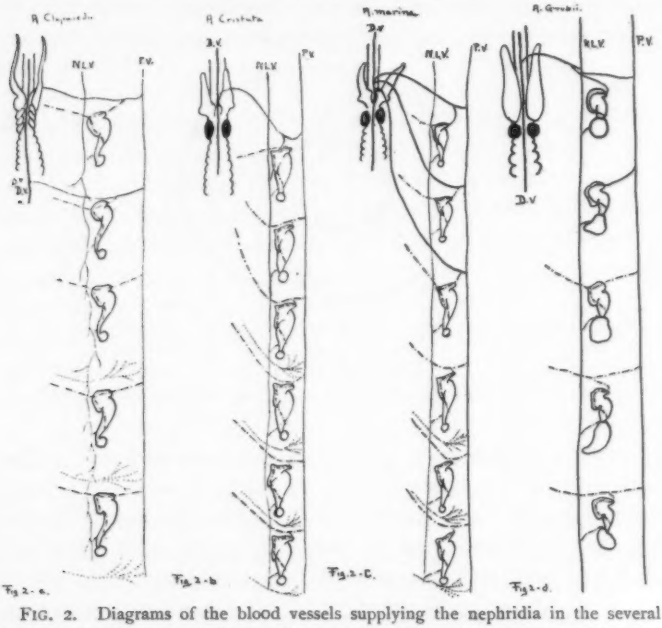


FIG. 2. Diagrams of the blood vessels supplying the nephridia in the several species of *Arenicola*, *a*, *A. Claparedii*, *b*, *A. cristata*, *c*, *A. marina*, *d*, *A. Grubii*; *d* will also serve as a diagram of the blood vessels of *A. ecaudata*. The form of the nephridia and of the digestive organs in the latter species, would be somewhat different but the arrangement of the blood vessels is the same as in *A. Grubii*. The dorsal vessel is shown by a solid line, thus ___. Afferent vessels arising from the ventral vessel are shown in broken lines, —.—.—. Efferent vessels connecting with the sub-intestinal blood vessel are shown in dotted lines, ... *D.V.*, dorsal vessel. *N.L.V.*, nephridial longitudinal vessel. *P.V.*, parietal blood vessel.

of previous authors. Thus in all nephridia of *A. Claparedii*, in some of *A. marina* and occasionally in *A. Grubii*, the afferent branch of the ventral vessel enters the *apex* of the funnel before it branches. In *A. cristata* one finds frequently, particularly in the somites containing the second and third nephridia, that the affer-

ent vessel gives off no branches to the nephridia, but the latter are supplied by a branch from the parietal.

The branch of the dorsal vessel to the second nephridium is small in *A. Claparedii*, but functional still. Gamble and Ashworth show and describe an afferent vessel to the first nephridium of *A. marina;* with this V. Willem disagrees, showing for this nephridium only a branch from the dorsal. In the majority of cases I find a branch from the ventral vessel also, thus agreeing with Gamble and Ashworth; in a few animals it is apparently wanting. I do not find, however, the efferent vessel from the fourth nephridium, in *A. marina*, to the subintestinal, which Gamble and Ashworth show but which V. Willem claimed was not present.

It is not true that the three main branches of the afferent vessel break up into capillaries; the one to the setal sac and gill does. The one to the integumentary vessels unites with them. The branch which enters the funnel continues as the gonadial vessel, runs peripherad to the nephridium and connects with the nephridial longitudinal, except in *A. Claparedii.*

The parietal and nephridial longitudinal vessels are distinct throughout the nephridial region in *A. Grubii*, *A. ecaudata* and *A. marina.* In *A. cristata*, the parietal is distinct but the nephridial longitudinal, while large at the level of the first nephridium, tapers posteriorly, becoming obscure back of the third nephridium. The parietal vessel is distinct the entire length of the nephridial region in *A. Claparedii.* The statement of Gamble and Ashworth that it is absent in this form is only explicable because they worked on preserved material; it is plainly evident in the living worm. The nephridial longitudinal, as a distinct vessel, is absent in this form except in the region of the first nephridium: its place is taken by a series of small connecting vessels, as if the gonadial vessel branched and some of its branches ran back to connect with those of the next posterior gonadial vessel.

In all cases the branches of the dorsal vessel running to the nephridia are afferent vessels. Gamble and Ashworth state that "The first nephridia of A. Grubii and A. ecaudata are *supplied* by a branch from the dorsal vessel" (the italics are mine), yet "The first three nephridia of A. marina, the first two of A. Clapa-

redii and the first of A. cristata *return* blood to the dorsal vessel." On *a priori* grounds, it would be strange to find the homologous blood vessels in closely related species carrying blood in opposite directions. I am certain that in *A. cristata* the blood flows from the dorsal vessel out to the parietal, the nephridial longitudinal vessel and to the nephridium, in this branch of the dorsal vessel. In this species the individuals are so large that in chloretonized specimens the direction of the blood movement is seen with comparative ease. S. E. Keith who has worked carefully for some time on the circulation of *A. cristata* gives me permission to quote her on this point as follows: "I have referred to these blood vessels in my own notes as the third pair of branches of the dorsal vessel. Gamble and Ashworth speak of the dorsal as receiving these branches, but the blood flows outward in them I am sure."

I have watched the blood-flow in *A. Claparedii;* the skin is frequently so transparent it may be seen without dissection. The flow is certainly away from the dorsal vessel in this species also. I am reasonably certain that such is the case in *A. marina*, in chloretonized specimens of which I have watched the blood movement. In this opinion I am supported by Willem who says of *marina*, "Il faut remarquer de plus, au point fonctionnel, que le sang circulant dans le tronc dorsal d'arriere en avant, le contenu des trois branches qui en emanent progresse dans une direction centrifuge."

Lillie makes the statement that "The vessel (segmental) begins its formation at the junction with the subintestinal blood vessel. Near its junction with the body wall the main vessel (segmental) gives off a branch (the nephrostomial vessel) which curves back, passes inward and barkward along the dorsal lip of the nephrostome." From this quotation it is evident that Lillie traces the afferent nephridial vessel to the subintestinal blood vessel, while, as stated above, I trace it, in agreement with Gamble and Ashworth and other observers, to the ventral vessel. The contradiction is only verbal, not real, for Lillie, in the explanation of his figures, labels this subintestinal vessel "the subintestinal *or* ventral vessel," a usage which he gets from oligochæt anatomy and which is incorrect here. Lillie finds that in *Arenicola* as in

other *Polychæta*, (see Fraipont on *Poygordius*, for instance), as well as in the *Oligochæta* (Wilson on *Lumbricus*), the first vessel to arise as a differentiation of the mesoderm, is the ventral vessel. Wilson says "The first vessel to appear (in *Lumbricus*) is the ventral or subintestinal." What the relation of this first vessel genenally is to the development of the circum-intestinal network in the polychæts is an unsettled point. (See Edward Myer's "Studien über den Körperbau der Anneliden," III., p. 464.) Recently Schiller writes as follows: "Nur bezuglich Blutsinus und Darmgefäsznetz ist mann bei *Arenicola Grubii* noch nicht ins klare gekommen, welches von den beider das primäre sei, da verscheidene Autoren ganz verscheidener Ansicht daruber sind. Die einer behaupten dasz zuerst der Sinus auftrete und sekundär sich in ein Netz auflöse: die anderen bezeichnen den Blutsinus als ein Produkt des zusammengeschmolzen Darmgefäsznetzes." He does not care to express an opinion on the subject although of the growing posterior region of *A. Grubii* he says: "Im Darmepithel keine besonderen Zellen vorkommen, die Antrit an der Bildung eines Sinus nehmen könnten." In considering der Blutsinus, Darmgefäsznetz und Subintestinalgefäsz he says: "Diese drei Gebilde werden hier zusammen als anatomisch und entwickelungsgeschichtlich zusammengehörende Elemente."

I have taken pains to quote in order to show that in spite of the difference of opinion regarding the origin of the network of intestinal vessels there is no tendency to include the ventral vessel in *Arenicola* or other polychæts as a part of this network which does, however, include the subintestinals. In *A. marina* we know (Benham, 1893) that the subintestinal vessels and the gastric longitudinals develop as the result of a fusion of the walls of the circular vessels of the alimentary canal. While in the oligochæts the subintestinal vessels split off from the ventral vessel. (Beddard, F. E., "Monograph of the Oligochæta," p. 70.) The term subintestinal vessel is an incorrect term for the vessels so named in *Arenicola*. Lillie should not have used the term subintestinal when he meant ventral, as these two terms are not interchangeable in *Arenicola*. The ventral vessel or subintestinal vessel of *Lumbricus* — they remain united in this form — is not homologous to the ventral plus the subintestinal vessels of *Arenicola*.

There is need of amendment in the description and figures of the form of the nephridia of the genus. The funnels, in particular, are much more symmetrical, in some species, than they have heretofore been shown. The usual technique is at fault in distorting them. The funnels are attached to the oblique muscles in *A. Claparedii*, *A. cristata* and *A. marina*. When the worm is opened and pinned out for dissection, the strain on these oblique muscles or on the connecting blood vessels of the other species, pulls the funnels throughly out of shape. It is, therefore, well to stupefy the worm by adding, drop by drop, seventy per cent. alcohol to the smallest quantity of sea water that will cover the worm in a long narrow dish. Then when the muscular walls are relaxed, the body cavity is injected by means of a hypodermic syringe or a fine pipette, until the walls are well distended with

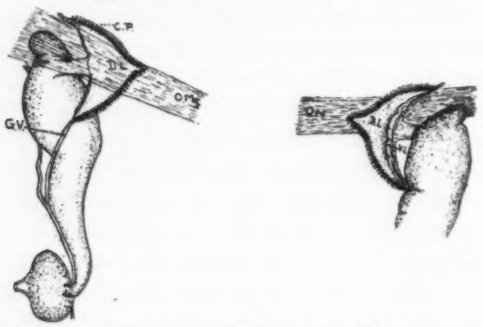


FIG. 3. Dorsal view of the second left nephridium of *Arenicola cristata*, × 3. *C.P.*, ciliated plates. *D.L.*, dorsal lip. *G.V.*, gonadial blood vessel. *O.M.*, oblique muscle.

FIG. 4. Ventral view of the anterior portion of second left nephridium of *Arenicola cristata*, × 3. *D.L.*, dorsal lip. *V.L.*, ventral lip.

the fixing agent. (Kopsch fluid answers well.) The whole worm is then placed in the fixing fluid. The nephridia are thus hardened without distortion and when dissected out later show their proper form.

The figures of the funnels or entire nephridia of each of the species are shown in the accompanying figures. The funnel of *A. cristata* (Figs. 3 and 4) is the most complicated and at the same time the most symmetrical in the genus. It is flattened, with a broadly sagittate or hastate form. Its dorsal lip,

which is attached by its outer surface to the oblique muscle, forms the point of the arrow. The ventral edge of this lip is set with from thirty to sixty ciliated plates which stand nearly at right angles both to the plane of the lip and to its edge. A loop of the blood vessel which traverses the dorsal lip runs up into each plate. The ventral lip is bow-shaped and divided into three segments like the handle and ends of a bow; the convexity of the bow turns toward the apex of the dorsal lip. The opening of the nephrostome is a long narrow slit between the base of the triangular dorsal lip and the bow-shaped ventral lip; the opening leads into the rapidly narrowing throat. The hastate funnel is held to the body by a short slender haft; the body of the nephridium, the glandular portion, is club-shaped; the funnel attaches to its side near the larger end, the axis of the

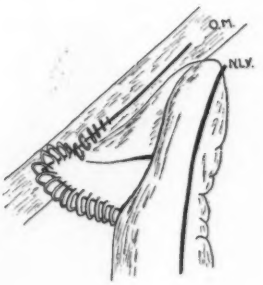


FIG. 5. Dorsal view of a nephridium of *A. marina*, × 15.

FIG. 6. Ventral view of the anterior end of the nephridium of *Arenicola marina*, × 15. *N.L.V.*, nephridial longitudinal blood vessel. *O.M.*, oblique muscle.

funnel being nearly at right angles to the longitudinal axis of the body. The body of the nephridium joins the roughly spherical bladder at the smaller end of the body, the handle of the club. The bladder opens to the exterior on its peripheral side by a very short duct leading to the nephridiopore.

The nephridium of *A. marina* approaches, at times, quite nea

to the form of that of *A. cristata;* but usually it is quite different from it (Figs. 5 and 6). The general shape of the body and of the bladder is nearly the same as in the nephridium of *A. cristata*, although in *A. marina* the body is more frequently curved while that of *A. cristata* is straight. The funnel shows great variation and often departs widely from the type of *A. cristata*. It is a flattened flap having roughly the shape of an equilateral triangle. At one angle the funnel opens into the anterior end of the body of the nephridium; along the opposite side lies the nephrostome. One of the sides adjacent to the neck is attached to the oblique muscle; the other adheres to the margin of the body. The axis of the funnel forms, therefore, an angle of only thirty or so degrees with the axis of the body. The dorsal lip is straight or slightly concave; it is set with twenty five or thirty somewhat lanceolate ciliated plates, each of which is supplied with a loop of the blood vessel. The ventral lip, Fig. 6, is regularly concave.

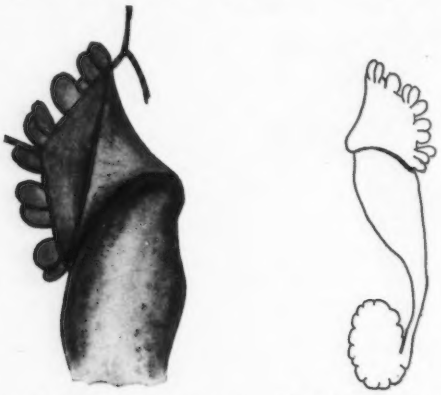


FIG. 7. Ventral view of the anterior portion of the third left nephridium of *Arenicola Claparedii*, × 30.

FIG. 8. Dorsal view of the nephridium of *Arenicola Claparedii*, × 15.

The ciliated plates which run along the edge of the dorsal lip, tend to continue along the blood vessel past the angle which the dorsal lip makes with the side of the funnel that attaches to the oblique muscle. This blood vessel runs along the muscle nearly parallel with the edge of the funnel. There are from ten to twenty of these plates; those along the blood vessel on the muscle

diminish in size. When occasionally there are an unusally large number of them along the blood vessel beyond the angle, the angle of the funnel adjacent to the muscle tends to become the apex of the funnel as in *A. cristata*, and the mouth shifts so as to more nearly face this angle instead of a side. In such cases the throat of the funnel opens into the body some distance back of the anterior end of the latter.

The funnel of the nephridium of *A. Claparedii* (Figs. 7 and 8) is least complicated. If we imagine a simple funnel form with short stem to be flattened and to have one lip pulled out into a triangular protrusion, we may gain a clear idea of the funnel of this species. The apex of the dorsal lip is broadly obtuse; the ventral lip is straight. The dorsal lip is set with the ciliated plates characteristic of the *marina* section of the genus which, as Gamble and Ashworth point out, includes the species *Claparedii*, *cristata*

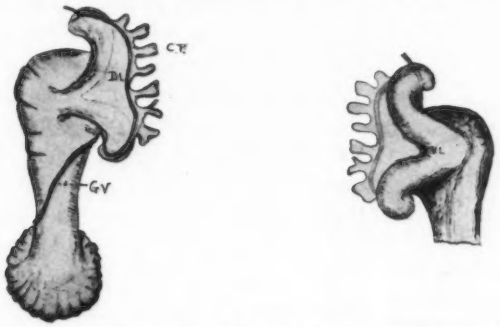


FIG. 9. Dorsal view of the first left nephridium of *Arenicola ecaudata*, × 15 *C.P.*, ciliated processes. *D.L.*, dorsal lip. *G.V.*, gonadial blood vessel.

FIG. 10. Ventral view of the anterior portion of nephridium (first left) of *A. ecaudata*, × 15. *V.L*, ventral lip.

and *marina*. There are ten or twelve of these plates in *A. Claparedii*. The funnel is at the anterior end of the body and its axis is also at right angles to the axis of the body of the nephridium.

The funnel of the nephridium of *A. ecaudata* (Figs. 9 and 10) is reniform in outline with revolute ends as seen from the ventral face. It also is flattened; the dorsal lip is slightly concave, the ventral lip is deeply indented. The dorsal lip is provided with ten to thirty blunt, at times much branched, finger-like processes;

these are covered with cilia, and within each is a blood sinus instead of a loop of the blood vessel. The throat of the funnel is relatively wide; the funnel attaches at the end of the body and usually has the customary position with its axis at right angles to the long axis of the nephridial body. Not infrequently however, the axis of the funnel is continuous with the axis of the body and we have a simple, unbent, tubular nephridium (Fig. 11). It is interesting to find such variation in this species for it makes evident how the more aberrant forms of the nephridium, such as occur in *A. marina*, are derived from a simple tubular type; just as a paper tube may be bent with its end at right angles to its major axis.

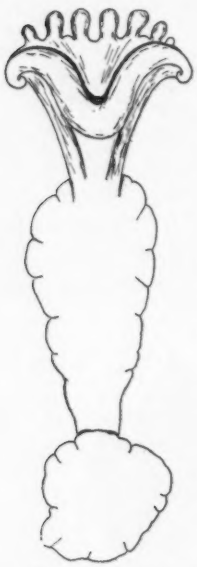


FIG. 11. An occasional form of the nephridium of *Arenicola ecaudata*, × 20.

A. Grubii (Figs. 12 and 13) possesses the same type of funnel as *A. ecaudata*. It is flattened; the dorsal lip is semicircular, the ventral lip trilobate, with a small median lobe and large lateral ones, thus making this lip deeply notched. Ten or twelve blunt, digitate, ciliated processes attach to the dorsal lip; these are often branched and are provided with the blood sinus. The funnel attaches to the body at some distance from the anterior end and its axis is at right angles to the axis of the body. The bladder of the nephridium of this species is usually expanded: it is capable of equally wide expansion in *A. ecaudata* but is more often found contracted. In the other species, the bladder is not so distensible although it is relatively large at times, especially when filled with eggs or sperm about to be discharged through the nephridiopore.

I have collected *A. cristata* and *A. marina* near Woods Hole, Mass., *A. marina*, *A. Grubii* and *A. ecaudata* in the bay at Plymouth, England, and have studied fresh *A. Claparedii* and *A. Grubii* at Naples. I wish to express my sincere thanks to the directors of the biological stations at these several places, who

have kindly placed at my disposal, material and facilities for the work, and to the Carnegie Institution whose table at Naples it was my pleasure to occupy.

FIG. 12. The nephridium of *Arenicola Grubii*, × 15, dorsal view. The outline of the ventral lip of the funnel is shown in dotted line.

FIG. 13. Ventral view of anterior portion of the third left nephridium of *A. Grubii*, × 20.

BIBLIOGRAPHY.

Benham, W. B.

'93 Postlarval Stages of Arenicola marina. Jour. Mar. Biol. Ass. Plymouth, N. S., Vol. III., pp. 48–52.

Eisig, Hugo.

'98 Zur Entwickelungsgeschichte der Capitelliden. Mitth. Z. Stat. Neapel, Vol. XIII., pp. 1–293.

Fraipont, J.

'87 Le Genre Polygordius. Fauna u. Flora d. Golfes von Neapel, Vol. XIV., Monographie.

Freudweiler, Hedwig.

'05 Studien über das Gefäszsystem niederer Oligochaeten. Jen. Zeitschr. f. Naturwiss., Vol. XL., pp. 1–28.

Gamble, F. W., and Ashworth, J. H.

'98 Habits and Structure of Arenicola marina. Quar. Jour. Micr. Sci., Vol. XLI., pp. 1–42.

'00 The Anatomy and Classification of the Arenicolidæ. Quar. Jour. Micr. Sci., Vol. XLIII., pp. 419–569.

Lillie, Ralph.

'06 Structure and Development of the Nephridia of Arenicola cristata, Stimpson. Mitth. Z. Stat. Neapel., Vol. XVII., pp. 348–405.

Meyer, Ed.

'01 Studien über den Körperbau der Anneliden, III. Mitth. Z. Stat. Neapel., Vol. XIV., pp. 247–585.

Schiller, Ignaz.

'07 Ueber den feineren Bau der Blutgefässe bei den Arenicoliden. Doctor's Thesis, Univ. Zurich, Jena.

Willem, Victor.

'99 L'Excretion chez l'Arenicola. Travaux de la Station Zoologique de Minereux, Vol. VII.

Wilson, E. B.

'89 The Embryology of the Earthworm. Jour. of Morph., Vol. III., pp. 388–462.

Vejdovsky, Franz.

'05 Zur Hämocöltheorie. Zeit. f. wiss. Zool., Vol. LXXXII., pp. 80–170.

SOME OBSERVATIONS ON THE HABITS OF PECTEN DISLOCATUS.

B. H. GRAVE.

With the purpose of studying the habits of the scallop, *Pecten dislocatus*, I collected many young specimens ranging from two to ten millimeters in length and placed them in small glass aquaria in the laboratory.[1] They were found in the harbor at Beaufort, N. C., well above the muddy bottom, clinging to eel grass. They were usually attached by several strands of hyaline byssal threads, which were exceedingly strong and elastic.

Although the *Pecten* is so generally known as to make a detailed description of its anatomy superfluous, yet a brief description of certain parts is deemed necessary. For a more detailed study of the anatomy, reference can be made to a paper, by G. A. Drew, on " The Habits and Anatomy of the Giant Scallop."

By reference to Figs. 1 and 2, it may be seen that the shell is rounded and eared. The ears make possible the long, straight hinge line, which extends along their upper borders to their extremities. The right valve is slightly more convex than the left, and near the anterior[2] ear, it has a deep notch. This one feature mars the symmetry of the valves. Between the valves and just beneath the hinge ligament, there is a pad of cartilage-like substance, which is compressed when the valves are closed. It serves to open them quickly when the adductor relaxes (Fig. 3).

The form of the shell and the structure and arrangement of the soft parts within, adapt *Pecten* to the swimming habit. It swims by opening and closing the valves in rapid succession. By varying the position of the mantle so as to control the direction of

[1] Through the courtesy of Hon. Geo. M. Bowers, U. S. Commissioner of Fish and Fisheries, I had the privilege of occupying a table in the Fisheries Laboratory, at Beaufort, N. C., for two months during the summer of 1908. For this privilege and for many kindnesses shown me by the Director, Henry D. Aller, I am glad to express appreciation.

[2] The hinge line is here considered dorsal for convenience in description, although it does not represent the true dorsal of the animal.

the currents of water expelled from the mantle chamber, it is enabled to swim either forwards or backwards, although it usually swims with the opening of the valves directed forwards.

A perfectly symmetrical shell is the form best adapted to swimming, and the presence of any irregularity in it, such as that just mentioned in the pecten, is to be explained either as an adaptation to habits other than swimming, or as a structure, inherited from an ancestral form, and not as yet obliterated through adaptation to the swimming habit.

Although adult specimens were kept in aquaria all summer, no method of locomotion other than smimming was noted, and no clue was gained as to habits which would in any way explain the function of the notch in the right valve. They neither attached themselves by a byssus, nor used the foot for locomotion. Young specimens, however, showed much more activity than the adults, and some observations on their habits are recorded in the following pages.

Concerning the function of the notch in a related species, *Pecten tenuicostatus*, Dr. Drew writes as follows: "I have been unable to satisfy myself as to the function performed by this notch. The sense tentacles on the mantle margin, opposite the notch, are somewhat longer than those adjacent, but I have been unable to determine that they have a special function or that they are especially advantageously placed."[1]

The Sense of Position.

The *Pecten* lies habitually upon the right valve and if placed upon the left, immediately turns over. When lying upon the left valve, it seems to feel the same sort of discomfort which a frog, or other animal with well developed balancing organs, feels when placed upon its back. However, after turning them over repeatedly, they sometimes remained resting on the left valve for several minutes.

The Asymmetry of the Valves.

When dropped through a considerable depth of water, *Pectens* settle about as frequently upon the left valve as upon the right.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 7.

The slight flatness of the left valve does not serve to make them settle always upon the same side.

The Functions of the Foot.

The foot lies just opposite the notch in the right valve. It appears to be functionless in adult specimens, or rarely used by them, but is made use of to good advantage by the young. Specimens were often seen to extend the foot anteriorly to a remarkable distance, attach it at the tip to the bottom and then, by a powerful contraction, draw the body forwards to the point of attachment. The foot is cylindrical and seems very small to carry such a load; and frequently, after it has been extended and attached, the valves are opened and clapped together, at the same time the foot contracts, the body thus being drawn forward with much less strain upon that organ. This method of locomotion is a combination of swimming and creeping. The force of the current of water expelled from the mantle chamber serves to raise the body and propel it forwards as far as the attached foot will permit. At the same time, the foot contracts and the body lands close to the point of attachment. When this method of locomotion is used, the foot, instead of being extended directly outwards, anteriorly, is usually directed more ventrally, so that the point of attachment is more nearly in line with the force exerted by the swimming movement. Except for the notch in the right valve, this sort of performance would not be possible, because by the closure of the valves, the foot would be crushed.

The foot is, also, frequently used in turning the body over, when placed upon the left valve; it is extended anteriorly from the body and attached; the valves are opened and clapped together vigorously; the body, as a result, is raised and shot forwards, but the weight of the foot and the resisting pull from its attachment cause it to swing over upon the foot as a pivot, the scallop landing upon the other valve, having turned through an arc of 180 degrees.

The above method of turning over, is usually, if not quite universally, used by specimens when placed upon the left side for the first time. After a little handling, however, they become much more irritable, seeming to be excited, and at such times, they

manage to right themselves by one of three methods: Sometimes without extending the foot, they open the valves and clap them together. After one or several trials, the body turns over upon the hinge line as a pivot. The mantle must have played a part in this by expelling currents of water in a direction such as to cause the body to turn over.

At other times, a position on the right valve is gained by one or more short swims, the method being continued until the body comes to rest on the right side. They usually manage to alight upon the right valve after a few trials, and then they become quiet.

The Byssus.

When specimens are allowed to lie undisturbed upon the right valve, they usually become attached by numerous strands of strong byssal threads. A short time only is required for this to take place. They frequently become firmly fixed in from two to five minutes and the threads are sufficiently strong to support a weight several times that of the body of the *Pecten*. The byssal threads pass through the notch in the right valve directly to the support below. They adhere, to some extent, to the shell where they come into contact with it.

So long as specimens are kept lying upon the left valve, they cannot, or do not, attach themselves by the byssus. Since the byssal gland lies at the base of the foot, it is possible that the notch, in the shell opposite it, is a structural adaptation directly correlated with the function of the byssus. At any rate, because the byssal threads extend through the notch, in place of over the edge of the shell, the pull has less tendency to tilt the body than would be the case if no notch were present.

In order for the byssus to become attached to the bottom, it is not necessary for the valves of the shell to be opened, since the attachment of the byssus is frequently accomplished while they are closed. The notch in the shell is sufficiently large to allow the extension of the foot to the support during the process of attaching the byssus. It seems that Dr. Drew has observed this process in individuals of *Pecten irradians*, to quote:

"An individual of *Pecten irradians* placed in a glass dish of sea water will sometimes protrude its foot from the shell, apply it

closely to the bottom of the dish and after a short time, slowly withdraw it, leaving a rather broad band of slightly yellowish material attached to the glass and connected with the foot by the byssal gland. This is not composed of small threads as in the mussels *mytilus* and *modiola*, but it may be sufficiently tough to support the weight of the animal, if, after a few minutes, the dish is carefully turned over."[1]

Summary.

By way of summary, therefore, it might be said concerning the function of the notch that it makes possible a much freer use of the foot and byssal gland, and is in some way connected with the function of these organs. Although many mollusks live in the mud, the fact that young *Pectens* do not is evidence that they do better out of it. The foot and byssus enable them to climb upon supports and maintain their position there. As they approach maturity, they assume more and more the swimming habit and the foot and byssus lose, to some extent or entirely, their functional activity. If these organs are not functional in full-grown *Pectens*, as seem probable, the notch is no longer of any value to them, although it is not obliterated.

The *Pecten* has the sense of position well developed.

Earlham College, Richmond, Indiana,
March 6, 1909.

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 18.

Explanation of Plate I.

Fig. 1. Shows the left valve as it appears from surface view. It is not quite symmetrical.

Fig. 2. Shows the right valve as it appears from surface view. The prominent notch at the base of the anterior ear can be seen.

Fig. 3. Is a view of the inner surface of the right valve. The dotted line shows the position of the cartilage pad which aids in opening the valves.

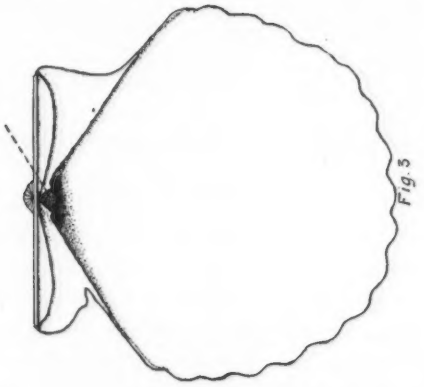


Fig. 3

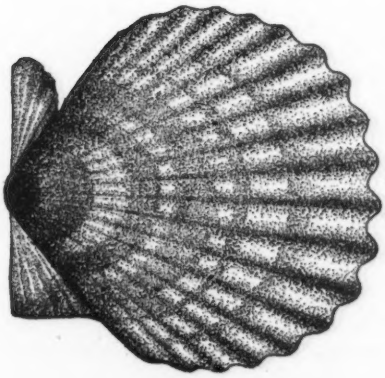


Fig. 2

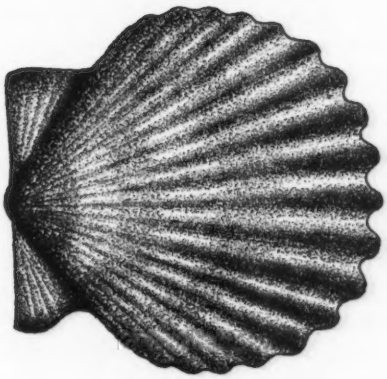


Fig. 1

of a basal hydranth. (*B*) The hydranth may produce some materials that inhibit the development of other hydranths from the remainder of the piece; hence the inhibition, as long as a hydranth is present or developing. On first thought these alternatives would seem to cover the only possible ways in which the problem of regeneration may be presented — at least as long as the problem is confined to purely physiological actions of a chemical order. There are, however, not a few considerations indicating that the fundamental interpretation may lie in a different conception of the problem. I shall try here to emphasize this other point of view without attempting to develop it into a theory of regeneration. At most we may hope at the present time to find in the facts some indication of the nature of the problem if not its entire elucidation.

A number of experiments have been made that seem to indicate that the temporary inhibition of the development of the basal hydranth in *Tubularia* is not the result either of the using up of materials by the oral hydranth, or of the setting free of inhibitory stuff. The simultaneous development of hydranths at both ends of a piece, which frequently occurs in short pieces, is a case in point. Both ends develop at the same rate as when a single hydranth develops, and not half as fast as the hypothesis demands. Again the development of a basal hydranth does not appear to inhibit the oral development as we should expect if the result were dependent simply on the presence of materials in the stem. Some experiments of MacCallum's with plants have an important bearing on this point. If the terminal bud of the bean is removed, the buds in the axils of the cotyledons develop. But if the activity of the terminal bud is simply lessened by inclosing that part in an atmosphere of hydrogen, the basal buds do not develop. Hence the result is due not to activity of the terminal end, but to its presence or absence. In a different way the same fact is brought out. A piece of willow stem is cut off, its middle third is inclosed in a tube filled with moist air, so that the buds in this part are encouraged to begin their development; the dry air retarding the development of those outside. After the middle buds have unfolded, the entire piece is inclosed in a moist chamber, when the more apical buds sprout forth, while none

of the buds basal to the middle region develop. The presence of growing shoots in the middle of the piece does not inhibit the apical buds from developing, if external conditions are supplied favorable to their growth, but the basal buds are inhibited by the presence of shoots on the more distal parts. These facts are incompatible with the assumption that the results are due to the presence of materials used up by those parts that develop first to the exclusion of other parts. They also show that the alternative view is untenable, for, the presence of growing shoots in the middle of the piece is not antagonistic to the development of shoots in other regions provided those regions are situated more distally.

In the case of *Tubularia*, it is more difficult to present convincing evidence that distal hydranths do not produce materials inhibiting the development of basal hydranths, improbable as such an interpretation may now seem. But the fact that basal hydranths do develop after the oral hydranths have formed may seem to discredit this view. Here, however, an apparent paradox is found. The experiments seem to show that when the oral hydranths develop, the basal hydranths are retarded in development, but they do develop later, and the results also show that if both start simultaneously both develop at the normal rate. The paradox is due, I think, to two antagonistic factors at work at the same time. Admitting that the oral development tends to inhibit the beginning of basal development, we also find that if other influences suffice to start both simultaneously, the on-rush, so to speak, of the process once begun changes the conditions that tended to prevent the starting. Strange as this seems it is little more than a statement of the facts. The same results may be put in a somewhat different way. A cut end being present, whether oral or basal — the conditions that call forth hydranth formation are given. Experiments show that the oral end tends to develop first, its development acts as a partial inhibition of the basal hydranth-formation. If this influence is strong enough the basal development is temporarily held in abeyance, but if not the inhibition is overcome. Once overcome, the formative influences do not check the further action of the basal end. In this connection it is curious to note that small oral pieces produce simultaneous hydranths more often than larger

pieces. The interpretation of this seems to be that the tendency to produce hydranths, both oral and basal, is stronger near the distal end and decreases basally. In short pieces the sensitiveness of the two ends to those influences that call forth the hydranth is so great that both ends develop simultaneously or nearly so, hence the oral end has not time to get a sufficient start over the basal to stop its development. It should be noted in passing that it is probable that the influence preventing basal development is not only the oral development, but a direction-factor present in the stem at all times. This factor we call polarity. The interesting point is that this factor seems to be more capable of inhibiting basal hydranth formation when an oral end is developing than when such development has not yet begun. The basal development, however, does not appear to delay the oral process. It is acting against the polarization and its influence is less felt throughout the stem, as experiments by Stevens and myself have shown. These considerations lead, I think, to the view that the essence of our problem lies in that peculiarity of the piece that we designate its polarity, and not in the absence or presence of formative substances in Sach's sense.

If our analysis is correct, we are led to look upon living material as possessed of a certain formative principle that has so to speak a "sense of direction." The next step will be to study the nature of this principle and see what properties we are justified in ascribing to it; for while it may be beyond our powers at present to state precisely the nature of the directive principle, we may at least be enabled to work out its manifestations. Some of these manifestations become apparent in the study of the regeneration of *Tubularia*. One of its most striking modes of action is seen in the inhibition of basal-hydranth formation. Most interesting is the result that its action becomes intensified by developmental processes going on at the oral end, as shown by the fact that if the oral development is suppressed by tying that end, the basal development is much accelerated. It is accelerated in the sense that basal hydranths more often develop at once than when both ends are open, but not in the sense that the basal development is faster than when this end also gets as early a start as the oral end. In other words, there is no speed-

ing up of hydranth formation as such, but the initial inhibition is overcome.

The special problem with which this paper deals is the nature of what takes place at the basal ends when the oral end is kept open and when it is tied. Is the retardation of such a kind that a slower process of development is going on at the basal end while the oral end is developing, or does the basal end not really begin to develop until the oral end has formed its polyps. If so, what gives it its start later? The following experiments were devised to study these questions.

Experiment I. — The purpose of the experiment was to determine whether when both oral and basal ends of a piece are left open constructive changes are slowly going on at the basal end. Some pieces were cut off and left open (*A*); later other pieces were cut off and the oral ends tied (*B*) and at the same time the oral ends of (*A*) were tied. It was found that the basal ends of the (*A*) pieces did not develop faster than those of the (*B*) pieces, showing that the changes at the basal end of (*A*) are not progressing, but are held in check by the developing oral hydranth.

Control I. — In some pieces the old hydranths were left intact and the pieces cut off. No basal hydranths began to develop until the old heads began to be absorbed. The presence of the old heads inhibited the development of the basal hydranths until the heads had degenerated when the latter appeared.

Experiment II. — In order to find out whether, when the oral end is tied, changes take place throughout the piece that tend to make more rapid the development of basal hydranths, or whether these changes are localized at the basal end where the new hydranth develops, the following experiment was tried. The oral ends of many pieces of the same length were tied. Then after several hours' interval differing in several experiments, the basal end was cut off, (*a*) just inside of the area that would form the basal hydranth, (*b*) in the middle of the piece, (*c*) just below the ligature. In general the development of the basal hydranth was delayed as compared with control pieces tied but not cut off at the basal end; the delay was the greater the further removed the cut from the basal end, despite the fact that oral levels tend to regenerate faster than more basal levels. The differences

are more apparent the longer the time that elapses before the basal pieces are removed. The differences are not very marked at the different levels indicating perhaps that changes take place throughout the piece and not only at the basal end although more pronounced in the latter. The different levels of the cuts make it difficult to ascribe the results solely to the general changes in the piece, for the more orally situated cut ends have an advantage in level as other experiments have shown.

Experiment III. — Pieces were cut off at the same oral levels. After 23 hours the hydranth region at the oral end was cut off of some pieces (*A*), others were cut in two in the middle of the piece (*B*), and for a control some pieces were left as before (*C*). A slight retardation occurred after another 12 hours in (*A*), less in (*B*) as compared with (*C*). Removal of the hydranth forming region after 23 hours causes delay but the delay is not so much as though a new hydranth had developed at the new cut, showing that changes directed towards hydranth formation are going on not only in the region where the hydranth will develop but at more basal levels as well.

Experiment IV. — This experiment was like the last, except that the basal ends of all the pieces were tied, thus preventing the basal end from exerting any influence on the result. Other experiments had shown, however, that the basal development, even if it occurs, has apparently no retarding influence on the oral development. The results, as was to be expected, were the same as in the last experiment. It is interesting to note that in both the influence of the cutting causes a greater delay in the first appearance of the primordia of the hydranth than on their later development; for later the differences seem to be less than at first. This may be due to an acceleration extending throughout the whole time that is more effective after a beginning has been made than before the start.

Experiment V. — Some previous experiments had left undecided the question whether, when the oral end is left open for several hours and is then tied off, the basal development is more rapid than when the oral end is tied at once. If such an acceleration really occurs it might seem to indicate that changes take place in the oral end that produce accelerating materials even for the

basal ends. I was particularly anxious to settle this point definitely, for obviously, if such acceleration could be proved, it would furnish evidence in favor of a chemical process, especially since other experiments had seemed to show that the basal end does not begin its development when both ends are left open. I have carried out rather an extensive series of experiments that give, I think, a definite answer to the question.

When pieces are left open at both ends from four to nine hours, and are then tied at the oral end, the basal development is slightly retarded as compared with its development in pieces tied at once. There is little evidence in favor of the view that the later tied pieces can make good the loss of four to nine hours, and of course they can not catch up if a longer time elapses. Whether they may do so in later stages is more difficult to decide, but this does not concern the main point here raised.

Individual differences in rate, differences in stems, and uncontrollable differences in level tend to obscure results that depend on only four, six, and nine hours differences in start. The above statement holds, therefore, only for average results. There was found no evidence in favor of actual acceleration, whether there is some relative acceleration is difficult to decide. If the hydranths do not develop promptly *i. e.*, if a long time elapses between the tying and the appearance of hydranths, the initial differences of a few hours may be lost.

Experiment VI. — Another attempt was made to see whether changes take place in the piece as a whole, after it is cut off, that make more rapid the development of an *oral* hydranth when a new cut is made.

Pieces were removed and after four hours somewhat more than the oral hydranth region was cut off. In some cases the newly cut ends developed as fast as did the hydranth in the small pieces cut off, but the latter may have been retarded by the operation or by the smallness of the pieces; yet in some cases the development of the newly cut ends was as rapid as in control uncut pieces. This result indicates that changes take place in the pieces behind the actual region of hydranth formation that lead toward the development of a hydranth.

Experiment VII. — In this case pieces first cut off were after

ten hours cut in two in the middle. Comparing the rates of development of the oral ends of the oral halves with that of the oral ends of the basal halves it appears that the latter are slower, but there is evidence that the retardation may be somewhat less than the ten hours difference in initial start. The basal halves in this experiment are also somewhat behind the basal halves in the last experiment, which seems to show that the general changes in the excised pieces that go towards oral development decrease from the cut ends inwards.

It has not seemed necessary to give the details on which these general conclusions are based. The nature of the case makes it difficult to obtain results as definite as one might wish, despite the precautions that were undertaken to make the conditions as uniform as possible. The general conclusion that changes take place in the piece as a whole, after its removal, that are in the direction of hydranth formation, seems fairly certain. Less certain perhaps is the evidence to show that when the oral end is tied similar changes take place in the pieces that accelerate basal development in regions beyond the hydranth forming region, but this conclusion too is, I think, quite probable. The nature of these changes is not revealed.

The Dynamic Factor in Egg-Development.

Students of the processes of regeneration have without exception made use of the term polarity to express a directive factor observable in their results, and to this factor is sometimes ascribed an active rôle as a controlling influence, at other times the term is used descriptively merely as a statement that the new structures are directed in the same way as the part removed. In both respects the word has been useful, however vague our conception of what polarity may be. Our analysis of the process has now gone sufficiently far, I think, to justify us in an attempt to come to closer quarters with the term. Without reviewing the opinions that have been expressed as to the nature of polarity, I shall try to contrast two views of its nature that seem to me to represent the two main lines that speculative thought has followed. It should be noted that the term is used equally by students of embryology and by students of regeneration. The former

finds axial relations in the egg — polarity, bilaterality, radial symmetry, etc.; the latter finds the new organs regenerated in definite relations to the old. To some observers the distribution or the stratification of the materials of the egg, has seemed a sufficient basis for the results referred to under the term polarity; to others it has seemed more probable that there exists in the egg an arrangement or structure that has axial relations from which result not only the depositions of the formed materials but also the nature of the action of the parts. Polarity is from this latter point of view not simply a passive structure, but a relation of the parts that directs the shifting series of changes that we call development. At one time one of these views has seemed more probable; at other times the other. The history of modern experimental embryology and regeneration shows the influence that these views have had on those who have followed the new work. In a general way the two views may be classed as the materialistic or chemical and the dynamic or physical conceptions of the developmental process. At present it seems to the writer that the evidence has been steadily pointing to the second of these contrasting views as the more probable. As far as the egg is concerned, the recent experimental work goes to show that the visible inclusions of the protoplasm (yolk, oil and other granules perhaps) are not the fundamental causes of the formative processes, although they may be needed in certain regions to carry out the future development of the structures that there appear. In regard to regeneration it has been evident for some time, that the specification or the differentiation (with its concomitant products), cannot be unreservedly utilized as a basis for an explanation of formative processes that take place. For example, if the gross materials or the differentiations of the head end of a planarian are the causes of that region being a head, it is inexplicable that when the head is removed it could regenerate a tail. There must be something else behind what we see that is responsible for the change that takes place. These and other considerations lead to the view that there exists a fundamental property of living matter that is the formative principle of development. On two former occasions, when attempting to analyze the results of regeneration in *Tubularia*, the author tried to account for the re-

sults of polarity on the basis of a stratification of the materials. Influenced at the time by recent results in experimental embryology that seemed to show that visible substances of different kinds in the egg are really responsible for the development of its parts, the same idea was applied to the problem of regeneration, despite the fact that I had on more than one occasion rejected the hypothesis of formative stuffs, in Sach's sense, as sufficient to account for the facts of regeneration. Yet a careful reading of the papers here referred to will show that I still held, though perhaps not always consistently, to the conception that back of these differentiated materials lay the real differentiating factors.[1] It now seems to me that the evidence, which at that time seemed so strongly to favor the idea of the importance of the grosser materials of the egg, is insufficient to establish its case, and that the important factors of development are dynamic properties of the bioplasm, rather than the formed products of the egg, or of the differentiated products of the adult animal. This statement does not mean that the visible products in the egg play no rôle in development. The evidence still shows that they may do so, but their rôle seems to be secondary, not primary.

The interrelation of the parts seems to be one of the most evident expressions of the fundamental formative influences. Several years ago a consideration of a number of results in regeneration led me to state that this relation might be expressed as a sort of tension. This view has been objected to on the ground that it does not appear to explain the matter any better than before. In a moment of doubt and in order to give the

[1] One further word of explanation. The rate of hydranth formation varies with the distance of the cut end from the original hydranth. I have spoken of this difference in rate as explicable on the assumption of the hydranth-forming materials decreasing toward the base, *i. e.*, away from the hydranth. It was unfortunate to have used the term hydranth materials, although I made sufficiently clear in the text that I did not mean to invoke the stuff-hypothesis in this connection. It is not entirely clear on what the difference in rate depends; most probably on the stem being less specialized as a store-house of food substance nearer the hydranth; probably also on some difference connected with the thickness of the walls with which the specialization may also be connected; possibly neither of these but some more fundamental characteristic is responsible for differences in rate. In any case it is not obvious that there is any connection between this difference in rate and the polarity of the piece. The latter is the same for all levels — the time it takes the piece to be remodelled seems to be referable to something else.

statement a meaning for those who believe no suggestion to be of value unless it refer the problem to ordinary properties of inorganic bodies, I suggested that osmotic pressure might be the cause of the tension differences in the parts. This was an unnecessary concession. The behavior of fluid crystals (according to Lehmann) shows that the formative changes can be accounted for on the basis of a tension exhibited by the molecules of the substance of the crystal. While the organism may not be put down as a fluid crystal, still we see that physical properties other than osmotic pressure and surface tension may play an all-important rôle in form-changes. It may be that a similar property is the cause of the formative changes in the organism. In any case, the facts that I had in mind suggested that tension of some sort is an important dynamic factor in development, perhaps *the* important factor. The facts still seem to me to indicate some such relation between the parts, and no one regrets more than I that we cannot "explain" the results even if my suggestion prove to be in the right direction.

Still later a consideration of certain facts of development led to the suggestion that two known properties of the organism — contractility and irritability — also play a very important rôle in embryonic and regenerative development. I shall not attempt to review here the argument which led to this point of view. How far and in what sense contractility and irritability are better expressions of the tension hypothesis, it is not easy to state. So far as contractility is concerned Lehmann's recent important paper on "Scheinbarlebende Kristalle"[1] shows the possibility at least of referring this property also to a condition of molecular tension. We are still too ignorant of the physical basis of irritability to make speculations in this direction profitable, but it may be well not to lose sight of this property of living matter in our attempts to analyze further the problem of development.

Stereometry of the Bioplasm.

Polarity implies difference in one direction. Every student of regeneration knows that in all three dimensions of space the same factor is present. Polarity is therefore only a part of the problem,

[1] *Biol. Centralb.*, XXVIII., 1908.

and so far as it draws attention away from the whole problem it seems best to substitute the term stereometry.

Sufficient evidence has accumulated, I think, to show that stereometry has a dynamic side — in so far as it is a result of the molecular factors that determine the relations of the parts to each other. A question of fundamental importance here presents itself. If the formed substances at each level are the products of the bioplasm, must not the bioplasm itself be stratified in nearly the same sense? It was this idea that I had in mind when I wrote in 1906: "If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials." But the point I overlooked was that there is no need to suppose that a heterogeneous network of bioplasm exists because the visible structure formed by it is different. The relation of the polarized material to the ends of the material (indeed to all its directions) suffices to account for the difference of level. In fact if the stereometry rests on a dynamic and not a statical relation of the parts this is the logical standpoint.

It has been suggested that irritability may be related to the dynamic factor of development.

The effects of irritability at any level may be realized through the chemical changes inaugurated. These chemical changes once started may, if enzymatic, thenceforward continue (unless checked by other chemical processes), independently of the factor that set them going.